Coumarins: Old Compounds with Novel Promising Therapeutic Perspectives

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Abstract: Natural as well as synthetic coumarins have recently drawn much attention due to its broad pharmacological activities. Many coumarins and their derivatives exert anti-coagulant, anti-tumor, anti-viral, anti-inflammatory and anti-oxidant effects, as well as anti-microbial and enzyme inhibition properties. The recognition of key structural features within coumarin family is crucial for the design and development of new analogues with improved activity and for the characterization of their mechanism of action and potential side effects. The different substituents in the coumarin nucleus strongly influence the biological activity of the resulting derivatives. Although some coumarins have been already characterized to evoke a particular biological activity, the challenge would be the design and synthesis of new derivatives with high specific activity for other pharmacological targets and define their mechanism of action to achieve new therapeutic drugs. The present review highligts the current progress in the development of coumarin scaffolds for drug discovery as novel anti-cancer agents. The major challenges about coumarins include the translation of current knowledge into new potential lead compounds and the repositioning of known compounds for the treatment of cancer.

Keywords: Coumarins, structure-activity relationship, lead compound, drug development.

BACKGROUND

Over a century ago, Crum-Brown and Frasser proposed that the physiological action of a substance was linked to its chemical composition and constitution [1]. In the last decades considerable progress has been made regarding the isolation, synthesis, pharmacokinetics, pharmacology and toxicology of coumarins. As most studies are unrelated, a comprehensive review of current literature would be a valuable contribution towards the discovery, development or resurgence of biologically active coumarin derivatives with application in diverse human diseases. The present review summarizes the key structural features of this family and its related properties, with particular emphasis on cancer.

From a chemical standpoint, coumarin (2*H*-1-benzopyran-2-one) is the parent compound of the coumarin family, a large class of naturally occurring phenolic compounds. Coumarin could be considered like the resulting fusion of benzene and a 2-pyrone ring. In nature, the heterocyclic ring is oxygenated at C-7 and less frequently at C-5, C-6 and C-8. These extra phenolic hydroxyls groups are sometimes derivatized as glycosides. The oxygenation patterns mentioned above are typical for benzenoid rings of C6-C3 units derived from the shikimic acid pathway. Compared with alkaloids synthesized through shikimic acid, there is a remarkably large number of compounds in which the nucleus is alkylated by one or more isoprenoid units [2]. In general, it can be established that this family of compound obey Lipinski's rule of five and exhibit cell membrane permeability, which are common characteristics found in most available drugs today [3].

Based on the substitution pattern, coumarins show anticoagulant, anti-tumor or antiviral properties whereas other derivatives behave as enzyme inhibitors or display antioxidant or anti-inflammatory properties. Although the coumarin system can be considered as one of the most important classes of heterocyclic compounds, based on *in vivo* experiments in rats, coumarin was banned from the market by the Food and Drug Administration in 1952. Since then a dispute over its toxicity has been raised [4]. Several reports point out that the toxicity of coumarin is metabolism and species dependent.[5, 6] Therefore, the evaluation of coumarin cytotoxicity in humans based on studies performed in rabbits or rats seems rather inapropiate. Several authors reported that coumarin compounds show no evidence of initiating tumors in different animal models [7]. Furthermore, coumarin and its derivatives are not mutagenic in the AMES or micronucleus tests[8, 9], and fail to exibit teratogenic properties[4].

Over the last 50 years coumarin compounds have been widely used as anti-coagulant, anti-microbial and anti-inflammatory agents supported by different clinical studies. Nevertheless, these compounds or their analogues have also emerged as promising drugs for cancer. In the present review selected examples will be discussed to illustrate the progress made in the development of natural and synthetic coumarins as potential anti-tumor agents.

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WARFARIN, FROM AN ANTI-COAGULANT TOWARDS AN ANTI-TUMORAL AGENT

Oral anticoagulants of the 4-hydroxycoumarin class, such as warfarin (Fig. **1A**), represent the most commonly prescribed drug for therapy and prevention of thromboembolic conditions for over 50 years. It was designed followed the identification of dicoumarol as the causal agent of a haemorrhagic disorder affecting cattle after consuming spoiled sweet clover hay [10]. Although the anticoagulants of the 4-hydroxycoumarin class exhibit high efficacy and are rather inexpensive, their narrow therapeutic index may sometimes complicate patient management [11].

4-Hydroxycoumarins inhibit vitamin K epoxide reductase (VKORC1) [12] leading to deficiency of vitamin K and subsequent deficiency of vitamin K-dependent proteins, including those involved in thrombus formation. The minimal structural requirements for the anticoagulant activity of the 4-hydroxycoumarin class, represented by warfarin, are an intact 4-hydroxycoumarin residue and a carbon chain in position 3 [13] Recently, Gebauer *et al*., demonstrated that the *in vitro* inhibition of VKORC1 requires deprotonation of the 4-hydroxycoumarin moiety whereas the substituent on carbon 3 modulates the inhibition, being more potent those derivatives with an isoprenyl side chain. Thus 4-hydroxycoumarins would bind to the active site of the enzyme mimicking a transition state [14].

Recent studies point to warfarin as a promising drug for cancer treatment. However, a few randomized trials have addressed the therapeutic efficacy of these anticoagulant agents in cancer [15]. Therefore, it is not possible to determine whether the possible benefit of anticoagulation results from an effect on the clotting system, a direct cytotoxic activity of the anticoagulant, or a change in the pharmacokinetics of the cytotoxic drugs caused by the anticoagulant. A study by McCulloch *et al*., suggests that warfarin may inhibit tumour metastasis without affecting growth rate of tumour cells *in vitro* at concentrations below 1 mM [16]. In accordance, Velasco-Velazquez *et al*., reported that 4-hydroxycoumarin, which lacks anticoagulant activity since it is unsubstituted on carbon 3, selectively disorganizes the actin cytoskeleton in a highly invasive melanoma cell line [17, 18]. These findings indicate that 4-hydroxycoumarin might be useful in metastasis and melanoma therapy. Furthermore, it highlights the fact that different molecular shapes are responsible for the anti-coagulant and the anti-tumoral activity.

Other studies suggest that dicoumarol [an anticoagulant coumarin; 3,3'-methylenebis(4-hydroxycoumarin)] (Fig. **1B**) and its analogues may inhibit cell proliferation by interfering with the spindle microtubule dynamics [19]. There is growing interest to design combinations of antimitotic coumarins and chemotherapeutic agents to improve efficacy and lower toxicity, such as taxol and dicoumarol, which results in a synergistic inhibition of cell division [20]. However, recent data published by Buey *et al*., showed that dicoumarol fail to stabilize microtubule in carcinoma cells [21].

Nowadays, there is renewed interest in determining whether anticoagulation therapy may improve the survival of oncology patients. In addition, the pharmacomodulation of anticoagulant coumarins have led to the development of novel analogues which inhibit the formation of experimental metastases.

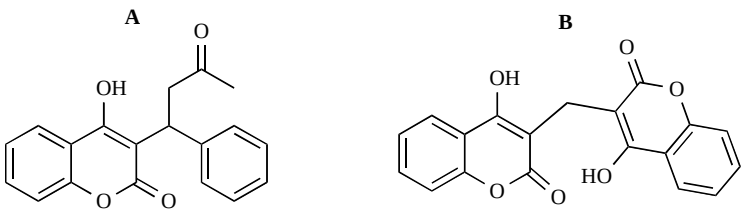
NOVOBIOCIN, FROM GRAM-POSITIVE BACTERIA TOWARDS CANCER CELLS

Although most of the natural coumarins have been isolated from plants, the aminocoumarin antibiotics novobiocin, chlorobiocin and coumermycin A1 were isolated from diverse *Streptomyces spp* and exhibit a potent activity against Gram-positive bacteria. These compounds target the bacterial enzyme DNA gyrase and inhibit the enzyme-catalyzed hydrolysis of ATP [22].

Novobiocin bears a carbamoylated sugar residue, a 3-amino-8-methyl-4,7-dihydroxycoumarin moiety (ring B) and an isopentenyl-substituted hydroxybenzoyl moiety (ring A) (Fig. **2**). Both, the ring B and the sugar residue are involved in ATPase inhibition at the B-subunit of DNA gyrase. Examination of the binding site of novobiocin reveals an extensive hydrogen bonding network, involving especially the novobiose sugar. It appears that the coumarin ring is crucial in directing the sugar moiety to the appropriate site whereas the ring A moiety would influence the uptake of the compound into bacterial cells [23-25]. Their poor oral absorption as well as their ability to develop resistance limit the use of aminocoumarins. In the past years several studies were conducted to design effective orally bioavailable coumarin antibiotic inhibitors of bacterial DNA gyrase [26-28].

Lately, the heat shock protein 90 (Hsp90) emerged as a promising target for cancer therapy [29] and novobiocin analogues gathered the attention of researchers since structure-activity relationship (SAR) studies showed that these coumarins bind to the Hsp90 C-terminal ATP binding site and induce degradation of Hsp90 client proteins [30-32].

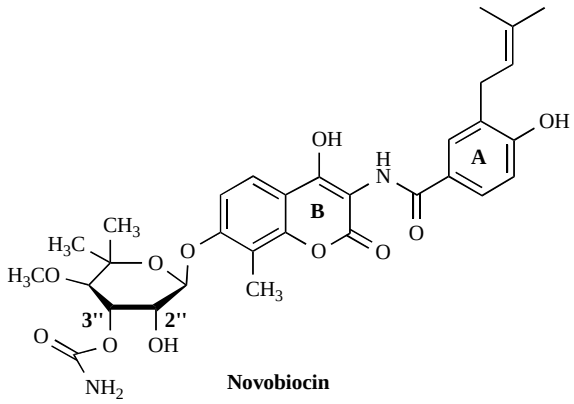
In order to establish coumarin compounds that could differentiate between the C-terminus of Hsp90 and DNA gy-



Warfarin
(3-(1-phenyl-3-oxobutyl)-4-hydroxycoumarin)

Dicoumarol
3, 3'-methylenebis(4-hydroxycoumarin)

Fig. (1). Warfarin and dicoumarol, the parent compounds of the anticoagulant coumarins.



Novobiocin

Fig. (2). Novobiocin, an aminocoumarin antibiotic.

rase, a library of novobiocin analogues was designed to convert a well-established gyrase inhibitor into a selective Hsp90 inhibitor. Studies show that the elimination of the 4-hydroxy group of the coumarin ring and the 3''-carbamate of the novobiose residue are necessary to achieve derivatives with a higher selective activity for the Hsp90 protein. These findings suggest that the 2'',3''-diol of the novobiose appendage, the novobiose moiety to the 7-position and an amide linker at the 3-position of the coumarin ring play a critical role for anti-Hsp90 activity [33, 34]. Donnely *et al.*, conducted an extensive work to further explore novobiocin derivatives with variations in the coumarin scaffold. These coumarin-derived motifs possess hydrogen bond acceptors placed at positions 5-, 6- and 8- of the coumarin ring and analogues bearing modification of the coumarin lactone. The authors showed that the secondary amide at the position 3 is required for the antiproliferative activity and substituents as *o*-propoxy and methoxy at 6- and 8-position, respectively, lead to an increased activity. The lactone moiety of coumarins may provide beneficial hydrogen bonding interactions with the binding pocket. However, these interactions may not be required to manifest antiproliferative activity, suggesting that the coumarin scaffold just acts like a connecting structure between the sugar and the benzamide motifs [35]. On the other hand, another studies demonstrated that the removal of the novobiose moiety in novobiocin together with the introduction of a tosyl substituent at C-4 or C-7 of the coumarins provides novel lead structures with a 1000-fold increase in activity and enhanced rates of cell death, by stimulation of the extrinsic apoptosis pathway due to the activation of caspases 7, 8 and cleavage of the poly-(ADP-ribose)-polymerase (PARP) [36, 37].

These are the first set of coumarins designed to target the Hsp90 protein for cancer treatment. However, further studies are needed to achieve improved analogues in order to confirm whether the coumarin structure is essential as scaffold for Hsp90 inhibition in cancer cells.

OLD AND NEW COUMARIN COMPOUNDS AS POTENTIAL ANTI-CANCER DRUGS

Cancer therapy depends on the type of tumor, its location and extension. Radiation and chemotherapy (e.g. apoptosis induction) are the most conventional therapeutic modalities used but they are frequently associated with the development of drug resistance and systemic toxicity. In the last decades alternative cancer therapies like differentiation therapy, angiotenesis inhibition and hormone or tyrosine kinase inhibition were developed.

Apoptosis-Inducer Agents

Chemotherapy is the treatment of cancer with anticancer drugs, and its main purpose is to eliminate cancer cells. Necrosis and apoptosis are two experimentally distinguishable mechanisms of cell death whereas the term cytotoxicity simply refers to the cell-killing property of a chemical compound without defining a specific cellular death mechanism. In the literature, a considerable number of reports show that diverse simple coumarins exert cytotoxicity in various cancer cell lines and experimental animal models of cancer. However, the mechanism through which most of these compounds induce cell death in these models remains to be established.

Among the simple coumarins with pro-apoptotic properties, esculetin (**1**) exhibits anti-proliferative effect by inducing apoptosis in human leukemic cells [38] or in 3T3-L1 adipocytes in a time-dependent manner [39]. Moreover, it enhances taxol-induced apoptosis in human hepatocellular carcinoma cells (HepG2) [40]. The treatment with 6-nitro-7-hydroxycoumarin (**2**), 8-nitro-7-hydroxycoumarin (**3**) or 3,6,8-trinitro-7-hydroxycoumarin (**4**) exerts a cytotoxic effect leading to cell death by apoptosis in different human cell lines [8, 9, 41, 42]. Scopoletin (**5**) causes apoptosis in HL-60 promyelocytic cells [43] and in human prostate tumor cells [44]. 7,8-dihydroxy-4-methylcoumarin (DHMC, **6**) induces apoptosis in A549 human non-small cell lung carcinoma cells and leukemic cell lines (U-937 and HL-60) in a dose-dependent and time-dependent manner, although in those cell lines different signal transduction systems would be activated [45, 46]. Despite the differences among the cell lines, the relationship between the structure and the activity is clear. Kolodziej *et al.*, reported that the high citotoxicity of coumarins depends on the existence of at least two polar aromatic functional groups [47]. These findings were further confirmed by other authors who showed that at least two polar groups in the benzene ring, particularly phenolic groups at positions 6,7 or 7,8 , are essential to induce apoptosis in tumor cell lines, whereas coumarin derivatives bearing the *ortho*-dihydroxy substitution exert a higher cytotoxicity effect in those cells [48, 49]. Likewise, the presence of two neighboring hydroxyl groups at positions 5, 6, 7 and 8 of the aromatic nucleus is necessary for the anti-inflammatory effect of hydroxycoumarins. [50-53]. In addition, the relative position of the *ortho* cathecol moiety in the benzenoid ring of the coumarin is an important feature for the anti-oxidant activity. [54, 55]

Ishihara *et al.*, performed a quantitative structure-cytotoxicity relationship analysis of twenty coumarin analogues in the human squamous cell carcinoma line (HSC-2) [56]. Different hydroxycoumarins with the *ortho*-cathecol arrangement which exerted the highest cytotoxicity effect in this cell line were studied. The authors found a highly significant correlation between the cytotoxicity concentration 50 values and the following descriptors: absolute hardness, ionization potential and highest occupied molecular orbital (HOMO) energy. This finding shows that the cytotoxicity of

certain hydroxycoumarins depends on the electronic properties of the molecule. Hardness and softness properties are important factors to estimate the cytotoxic activity of coumarin derivatives.

Most plant-derived polyphenolic anti-oxidants may under certain conditions act as pro-oxidants and generate ROS thus behaving as cytotoxic and pro-apoptotic agents [57-59]. In this sense, flavonoids with the *ortho*-dihydroxy moiety are able to inhibit lipid peroxidation and scavenge superoxide but they also behave as pro-oxidant agents [60]. Studies by Paya *et al.*, showed that the dihydroxylated coumarins fraxetin (**7**), esculetin (**1**), 4-methylesculetin (**8**), daphnetin (**9**) and DHMC (**6**), are not only effective inhibitors of Fe^{3+}-ascorbate-dependent microsomal lipid peroxidation and aqueous alkylperoxyl radicals, but also scavengers of superoxide anion radicals [61, 62]. However, coumarins with *ortho*-dihydroxylation enhance hydroxyl radical generation in the Fe^{3+}–EDTA–H_2O_2 deoxyribose system, but decrease it in the Fe^{3+}–ascorbate–H_2O_2 deoxyribose system, supporting that they can chelate iron ions and also donate electrons, promoting a Fenton type reaction. These findings support that hydroxylated coumarins may either behave as ROS scavengers or pro-oxidant compounds depending on factors such as excess of free transition metal ions, metal reducing potential, metal chelating behavior, pH or solubility. It was reported that 7,8-dihydroxylated coumarins fail to act as cytotoxic agents but behave as scavengers of superoxide anion radicals. However, in the presence of free ferric ions they may exert potentially damaging pro-oxidant actions, including cytotoxicity. Conversely, 5,7-dihydroxycoumarin-4-methylcoumarin (**10**) inhibits lipid peroxidation and scavenges alkylperoxyl radicals but fails to display pro-oxidant activity [61, 62].

HL-60 cells exposed to scopoletin (5) undergo apoptosis that is prevented by an anti-oxidant suggesting that ROS generation is involved in scopoletin-induced apoptosis [43]. In agreement we reported a close relationship between the ability of hydroxycoumarins to induce apoptosis in leukemic cells (U-937 and HL-60 cells) and ROS generation. In terms of SAR, the existence of two adjacent phenolic hydroxyl grups is the most relevant factor, whereas the position of the *o*-dihydroxyl groups in the aromatic nucleus has little effect [49]. Similar SAR results were previously reported for hydroxycoumarins as inducers of Cu^{2+}-dependent DNA strand breakage [63]. We further reported that the methylation of the 6-OH group reduces the pro-apoptotic activity, being the reduction higher for monohydroxy-coumarins. Derivatives where the phenolic hydroxyl group is replaced by an amino, methoxy or methyl group fail to exhibit pro-apoptotic activity in U-937 cells. The presence of a methyl or hydrogen group at position 4 of the coumarin ring in most of the derivatives does not influence their pro-apoptotic activity. The presence of a hydroxyl group at position 3 in the pyrone ring does not display pro-apoptotic activity in leukemic cells [49]. Our findings support that DHMC (**6**) increases ROS and generates a phenoxyl radical as measured by ESR spectroscopy in U937 cells, indicating that the increased oxidative stress induced by DHMC (**6**) causes cell death. Furthermore, in U-937 cells pretreated with the radical scavenger N-acetyl-L-cysteine (NAC), DHMC (**6**) fails to induce DNA fragmentation and to trigger apoptosis.

In agreement with our results supporting the dual role of hydroxycoumarins as pro-oxidant and anti-oxidant agents, other polyphenolic compounds bearing free phenol groups, such as curcumin, resveratrol or epigallocatechin-3-gallate (EGCG) were shown to act as antioxidants at lower doses and pro-oxidants at higher doses under certain circumstances [64]. These molecules can participate in electron transfer reactions. It has been described that they may reduce ferric iron to ferrous iron, which can catalyze Fenton reactions and lead to the generation of the highly reactive hydroxyl radical. These chemical reactions disrupt mitochondrial redox homeostasis and induce mitochondrial-mediated apoptosis in various tumor cell types [65-68]. Similar results have been described for 2-methoxy-4-(2-propenyl)-phenol (eugenol) [69] or 2-(3,4- dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one (quercetin) [57]. Moridani *et al.*, reported a correlation between polyphenol toxicity and their lipophilicity (log *P*) in addition to phenoxy radical formation marked by the electronic Hammett parameter ($\sigma+$) and the OH homolytic bond dissociation energy [70]. Current evidence points to the coumarin ring as a modulator of the free hydroxyl groups to induce pro-oxidant effects.

Despite the numerous studies, the mechanisms underlying the beneficial effect of coumarins in cancer still remain to be fully elucidated. Our studies provided a clear relationship between ROS generation and the pro-apoptotic activity of hydroxycoumarins [49]. It is well established that ROS may stimulate and inhibit distinct signaling pathways, being the net result of ROS generation highly dependent upon the nature of the oxidative stressor and its cellular location [71]. Several researchers focused on different intracellular pathways in an attempt to elucidate the mechanisms triggered by coumarins in cancer cells. It is possible that exposure to selected coumarins might bring about significant cellular stress, resulting in a modulation of different intracellular pathways leading to cell death. However, whether other mechanisms are involved in the response it is presently unknown.

We recently reported that following 24 h treatment, DHMC (**6**) induces selective apoptosis in leukemic cells through the activation of the JNKs pathway and inhibition of the ERK1/2 pathway without contribution of the p38-MAP kinase cascade, members of the mitogen-activated protein kinases superfamily (MAPK). In addition cells exposed to DHMC for 18 h showed inhibition of the PI3K/Akt pathway, an important survival pathway in leukemic cells. Furthermore, down-regulation of c-myc protooncogene and induction of the cell cycle inhibitor $p21^{WAF1/CIP1}$ through a p53 independent mechanism was also observed. In these cells NAC pre-treatment delayed c-Myc and $p21^{WAF1/CIP1}$ expression, suggesting that these cellular pathways may be regulated by DHMC-induced oxidative stress [46]. Nevertheless, Goel *et al.*, showed that DHMC (**6**) caused apoptosis in human non-small cell lung carcinoma cells providing evidence that DHMC (**6**) induces apoptosis through a ROS independent mechanism by downregulation of Bcl-xl, Bax, p21, p53, Cox-2, ERK/MAPK and upregulation of c-Myc [45].

A recent study indicates that esculetin (**1**) enhances arsenic trioxide-induced apoptosis in U-937 promonocytic leukemia cells, but the response is reduced by NAC pre-

treatment. The authors propose that esculetin modulates MEK/ERK and JNK pathways and decreases intracellular reduced-glutathione levels, leading to a higher oxidative stress which would enhance arsenic trioxide-induced apoptosis [72]. A similar mechanism was observed in 3T3-L1 adipocytes [39]. On the other hand, in human renal carcinoma cells 6-nitro-7-hydroxycoumarin (**2**) induces apopotosis by sustained activation of p38-MAPK whereas 7-hydroxycoumarin (**11**) by activation of ERK1/2 without affecting p38-MAPK or JNK cascades [41, 73].

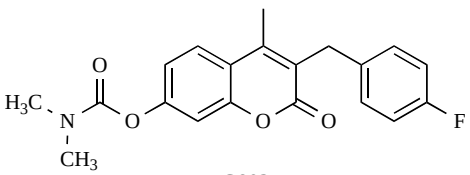
In the present review we described several studies where coumarin compounds bearing polar groups modulate members of the mitogen-activated protein kinase family. The underlying mechanism of coumarin-induced changes in the activation of MAPK cascades remains presently unknown but it is likely that coumarins may act upstream MAPK cascades. In this sense, it was reported that the anti-inflammatory and anti-cancer properties displayed by various coumarin derivatives result from an allosteric MEK1 inhibition, blocking ERK1/2 phosphorylation with no changes in total ERK1/2 levels. These novel MEK1 inhibitors are 7-aminocarbonyloxy-coumarins (named **G8935** and **GC63**) (Fig. **3**). Those coumarins were successfully docked into the allosteric site of the MEK1 structure, showing that **G8935** overlaps with PD3180088, a known MEK inhibitor. The carbamate moiety at C7 position, the carbonyl oxygen from the coumarin ring and the benzyl group at C3 seem to be essential requirements for the activity of these coumarins as MEK1 inhibitors [74]. As the coumarins bearing an *ortho* cathecol group mentioned as inducers of apoptosis in the present review, do not share the structural requirements described by Han *et al.*, they are likely to cause oxidative stress leading to the inhibition of survival cascades such as ERKs and PI3K/Akt, as previously described for other cellular types [75, 76].

In addition, coumarins are also involved in the inhibition of other protein kinases, Yang *et al.*, studied the effect of five mono- and di- hydroxycoumarins [77] and found that only daphnetin (**9**) inhibits the activity of serine/threonine-specific protein kinases, such as EGF receptor tyrosine kinase, protein kinase C (PKC) and cAMP-dependent protein kinase (PKA), which are implicated in cell proliferation, differentiation and death. In an attempt to establish a relationship between the structure and the inhibitory activity, it was concluded that the hydroxylation at C8 would be a structural requirement for daphnetin to act as a protein kinase inhibitor.

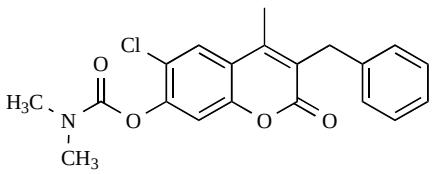
Other studies indicate that dihydroxycoumarins, such esculetin (**1**) and DHMC (**6**), or mono-hydroxycoumarins, such as scopoletin (**5**) and 7-hydroxycoumarin (**11**) inhibit cell cycle progression in different cell lines by inducing arrest in the G1 phase caused by an up-regulation of G1 associated cyclin-dependant kinase inhibitor $p21^{WAF1/CIP1}$, a downregulation of cyclin D1, an up-regulation of p27 and hypophosphorylation of retinoblastoma protein [46, 73, 78-81]. These findings support that blockade of G1 phase occurs following hydroxycoumarin-treatment, which ultimately is necessary for cell death. Considering that most used anti-neoplastic drugs induces cell cycle blockade in the S or G2/M phase, cancer therapy would be improved by combination of these drugs with coumarins that block the G1 phase. In addition, it has been reported that 7-hydroxycoumarin and coumarin itself cause a reversible inhibition of ras- and myc-induced neoplastic properties in transformed fibroblasts and in the MTV-EJras cell line [82, 83].

The selective tumor cell-specific cytotoxicity of coumarins has also been well documented [8, 42, 46, 48]. Finn *et al.*, showed the selective cytotoxicity of 6-nitro-7-hydroxycoumarin(**2**) and daphnetin (**9**) in human renal carcinoma cells, relative to non-carcinoma proximal tubular cells [8]. Other studies demonstrate that 6-nitro-7-hydroxycoumarin (**2**) and 3,6,8-trinitro-7-hydroxycoumarin (**4**) exibit high cytotoxicity in a melanoma cell line and reduce cytotoxicity in a normal fibroblastic skin cell line [42]. In accordance, we reported that DHMC (**8**) exerts significant less cytotoxic effect in normal mononuclear cells after 24 h treatment than in leukemic cells [46]. 7-Hydroxycoumarin (**11**) displays anti-proliferative effects in malignant cell lines but not in human peripheral blood mononuclear cells and human bone marrow progenitor stem cells at concentrations lower than to 200 μg/ml [84]. Kawase *et al.*, proposed that the tumor-specific cytotoxicity of esculetin (**1**) can be further enhanced by proper substitutions at 3- and/or 4-position(s) of the molecule [48]. However, the underlying mechanisms of the tumor-selectivity of coumarins are not well understood yet. Interestingly, cell malignization is often accompanied by a decrease in activity of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase), which increases the cell sensitivity to pro-oxidant compounds [85, 86].

In the last years the potential application of coumarins with metal complexes as cyto-selective therapeutic agents for cancer therapy gained growing interest. Complexes of coumarins with lanthanum(III), zirconium(IV) or cerium(III) represent interesting metalorganic compounds with antitumor activity in different cell lines [19, 87-90]. The cytotoxicity of the lanthanum complex of bis-coumarins in the chronic myeloid leukemia cell line is partly mediated by the stimulation of programmed cell death whereas the inorganic salt exerts a very weak cytotoxic effect [90]. Thati *et al*., demon-



G8935
3-(4-fluorobenzyl)-4-methyl-2-oxo-2*H*-chromen-7-yl dimethylcarbamate



GC63
3-benzyl-6-chloro-4-methyl-2-oxo-2*H*-chromen-7-yl dimethylcarbamate

Fig. (3). Coumarin derivatives with MEK1 inhibitor activity.

strated the anti-proliferative effects of a series of silver(I) complexes of coumarin-3-carboxylic acid derivatives using human-derived carcinoma cell lines. The authors concluded that hydroxylation particularly at position 6 and complexation with silver are structural requirements for the execution of apoptotic cell death [91, 92].

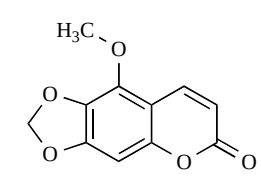
Differentiation Inducer Agents

Another potentially less toxic approach to treat cancer employs certain chemicals to induce differentiation of neoplastic cells. This approach fostered the concept of treating tumors by forcing malignant cells to undergo terminal differentiation instead of being killed through cytotoxicity. It is based on the assumption that many neoplastic cell types exhibit reversible defects in differentiation, which upon appropriate treatment, resulting in tumor reprogramming and a concomitant loss in proliferative capacity and induction of terminal differentiation [93, 94].

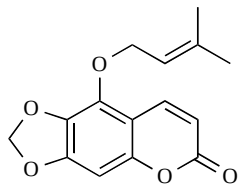
Several coumarin derivatives induce differentiation of human neoplastic cells. Daphnetin (**9**) exerts potent anti-proliferative and differentiation effects in a human renal cell carcinoma line [95]. Furthermore, esculetin (**1**) and 4-methylesculetin (**8**) differenciate HL-60 cells to mature monocyte/macrophage cells [96]. It was also shown that esculetin (**1**) significantly enhanced retinoic acid or DMSO-induced differentiation in HL-60 cells [97].

We reported that two pure trioxygenated coumarins, 5-methoxy-6,7-methylenedioxycoumarin (**C-1**) and 5-(3-methyl-2-butenyloxy)-6,7-methylenedioxy coumarin (**C-2**) isolated from *Pterocaulon polystachyum*, have anti-proliferative and differentiation properties in U-937 cells (Fig. **4**) [98]. These promising findings prompted us to investigate the anti-leukemia activity of a broader range of related polyoxygenated coumarins. Thus related natural and synthetic coumarins, including a variety of 5-substituted-6,7-methylenedioxycoumarins easily obtained by newly developed synthetic methods, were evaluated to identify the key structural requirements to induce differentiation in leukemic cells [99-102]. We found that the treatment with 5-(2-hydroxy-3-methoxy-3-methylbutoxy)-6,7-methylenedioxy-coumarin (**D-2**) and 5-(2,3-dihydroxy-3-methylbutoxy)-6,7-methylenedioxycoumarin (**D-3**) inhibit cell growth and induce the differentiation of U-937 cells after 48 h treatment (Fig. **4**). These results provide fruther insights into the correlation between some structural properties of polyoxygenated coumarins and their *in vitro* leukemic differentiation activity, showing that only 5-substituted-6,7-methylenedioxycoumarins display anti-proliferative and differentiation activity. Derivatives lacking an alkoxy group at position 5 or the 6,7-methylenedioxy arrangement fail to induce U-937 cell differentiation [102]. It is important to note that if the methylendioxy substituent is replaced by a furan group, the resulting 3,2-g-furanocoumarin loses the differentiation activity on leukemia cells and exhibits relevant applications in photochemotherapy for the treatment of psoriasis and other dermatological diseases [103-105].

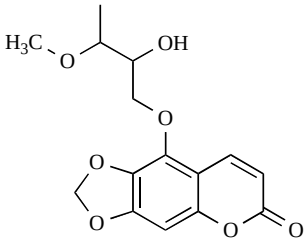
The mechanisms underlying the effect of 6,7-methylenedioxycoumarins in leukemic cell proliferation and differentiation are presently unknown. Coumarins such as esculetin (**1**) act as a differentiation agent by modulating 5-lipoxygenase metabolism [97]. Finn *et al.*, showed that p38-MAPK mediates the effect of daphnetin (**9**) in human renal cell carcinoma [95], although it has been described that daphnetin (**9**) can also inhibit EGF receptor tyrosine kinase, PKC and PKA activities, which have a relevant role in the control of cell proliferation, differentiation and metabolism [77]. The key molecular target of this group of compounds has to be identified in order to facilitate the development of new pharmacological tools with potential differentiation activity for the management of cancer. This may be useful to improve combined therapies, especially because they often have few side effects.



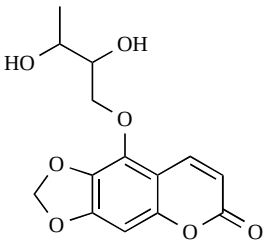
C-1
5-methoxy-
6,7-methylenedioxycoumarin



C-2
5-(3-methyl-2-butenyloxy)-
6,7-methylenedioxy coumarin



D-2
5-(2-hydroxy-3-methoxy-3-methylbutoxy)
-6,7-methylenedioxycoumarin



D-3
5-(2,3-dihydroxy-3-methylbutoxy)-
6,7-methylenedioxycoumarin

Fig. (4). 5-oxygenated-6,7-methylenedioxycoumarins with differentiation activity in human leukemic cells.

Hormone-Dependent Tumor Inhibitors

Briefly, we will mention a group of tricyclic coumarins designed as part of a programme to identify potent non-estrogenic steroid sulfatase inhibitors. Comprehensive reviews on steroid sulphatase inhibitors have been recently published [106, 107]. The development of inhibitors for the production of 5-androstenediol and estrone from sulfated precursors represents a new therapeutic approach for the treatment of hormone-dependent breast cancer. Studies with diverse tricyclic coumarin sulfamates tested for their ability to inhibit estrone sulfatase activity (E1-STS) showed that COUMATE (4-methylcoumarin-7-O-sulfamate) acts an E1-STS inhibitor in MCF-7 cells. 667 COUMATE (6-oxo-8,9,10,11-tetrahydro-7H-cyclohepta-[c] [1] benzopyran-3-O-sulphamate), not only inhibits STS activity but it also inhibits carbonic anhydrase II activity and behaves as a weaker aromatase inhibitor [108-111]. Encouraging results from a phase I trial show that 667 COUMATE (STX64) is a potent and well-tolerated STS inhibitor. It inhibits STS activity in peripheral blood lymphocytes and breast tumor cells, leading to a significant decrease in the serum concentration of steroids with estrogenic properties [112]. It has been suggested that STS inhibitors may also have a role in the treatment of other hormone-dependent cancers including those of the endometrium, ovary and prostate [106]. *In vivo* studies showed that coumarin itself strongly inhibits the growth of prostate tumours and DMBA-induced mammary carcinomas in rat. In addition, it also reduces the number of lung and lymph node metastases formed by the R3327-MatLu prostate tumor [113-115]. Nevertheless, the mechanisms of the antineoplastic and antimetastatic effects of coumarins *in vivo* have not been fully elucidated.

Multidrug Resistance Reversal Agents

Multidrug resistance (MDR) is a major complication in cancer therapy. One of the main causes of failure in cancer chemotherapy is the over-expression of P-glycoprotein (Pgp), an ATP-driven membrane exporter of hydrophobic xenobiotics, including anticancer agents. Therefore, modulation of Pgp has gained a great interest lately in cancer research [116, 117].

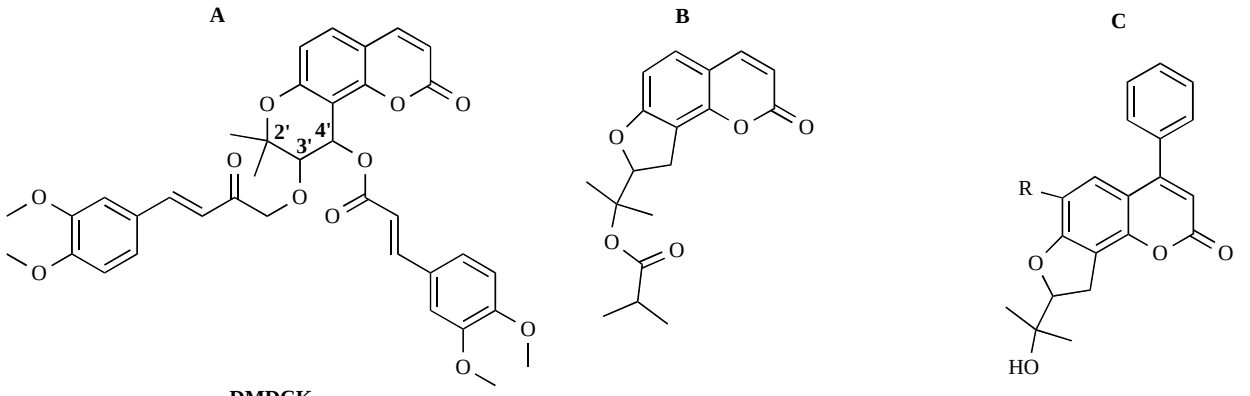
Several furanocoumarins, such as bergamottin (5-[(3,7-dimethyl-2,6-octadienyl)oxy]-furanocoumarin) and their derivatives have been reported as inhibitors of Pgp activity [118, 119].

Furthermore, (±)-praeruptorin A (**PA**) [(±)-3´-angeloyl-4'-acetoxy-cis-khellactone], a naturally occurring 7,8-pyranocoumarin abundantly found in *Peucedanum praeruptorum* Dunn., suppresses Pgp expression and reverses Pgp-MDR in KB V1 cells [120]. In an attempt to develop novel Pgp inhibitors, a number of PA derivatives were synthesized and a SAR study performed. **DMDCK** [(+/-)-3 '-O,4 '-O-bis(3,4-dimethoxycinnamoyl)-cis-khellactone)] (Fig. **5A**), bearing two 3',4'-dimethoxycinnamoyl groups, resulted the most effective Pgp inhibitor of the series. **DMDCK** is not a transport substrate of Pgp but it is an effective inhibitor of Pgp-mediated transport, suggesting a non-competitive mode of inhibition [121]. A pharmacophore group search was performed using the verapamil-based template as a model for Pgp substrates or inhibitors. This model involves two essential hydrophobic planes, three optional hydrogen bond (HB) acceptor points and one optional HB donor point. Both stereoisomers of **DMDCK** had four functional groups (two hydrophobic points and two HB acceptor points) simultaneously involved in the interaction with Pgp, implying a higher binding affinity and Pgp modulating activity. Results of the pharmacophore search provide an explanation on structural bases for MDR reversing activity of these pyranocoumarins derivatives [121, 122]. Furthermore, pyranocoumarins are as effective as verapamil, a calcium voltage channel blocker, in enhancing doxorubicin accumulation. **PA** was also reported to act as a calcium channel blocker, but further studies are needed to gain insight into the mechanism of pyranocoumarins [123, 124].

A 3D-quantitative structure-activity relationship was performed to evaluate the ability of a series of natural and synthetic coumarins to reduce the Pgp-mediated drug efflux of daunorubicin in human leukemic cells (K562/R7) overexpressing Pgp. The inhibitory activity was enchanced by the substitution at position 4 with a phenyl group, as supported by a 3D-QSAR analysis showing that a hydrophobic bulk group is favorable in that position of the nucleus. The importance of some substituents particularly dihydrofuranic moieties at positions C7–C8, which confers favorable electrostatic and steric effects for the activity, was also demonstrated. Acyclic substituents (i.e., acyl, prenyl and 2-hydroxy-3-methylbut-3-enyl residues) at position 6 or 8 only produce slight variations in the inhibitory activity of Pgp [125]. In other SAR study using 10 analogues of 4-phenyl coumarin, the authors confirmed the structural requirement in the aromatic ring of the [α-(hydroxyisopropyl) dihydrofuran] substructure with a positive effect due to steric considerations. They further described that the presence of methoxy groups at positions 5 and 7 also impacts on the Pgp inhibition (Fig. **5C**) [126]. In addition, the substitution of the lactonic ring by a hydrophobic moiety, like a 3-α,α-dimethylallyl group, also increases the inhibitory activity [127]. This SAR study confirms previous results reported for cnidiadin (Fig. **5B**), a furanocoumarin with a [α,β-di(hydroxyisopropyl)-dihydrofuran] group at positions C7–C8, which exhibits an anti-MDR activity in the MDCK-MDR1 cell line [128]. It should be noted that cnidiadin was evaluated with umbelliferone (**11**), esculin (**12**), esculetin (**1**), angelicin (**13**) and psoralen (**14**) and it was the only tested coumarin to competitiverly inhibit the binding and efflux of drugs by Pgp in the MDCK-MDR1 cell line.

A SAR study with 44 coumarin compounds was carried out by Kawase *et al.*, to identify the basic features of coumarin structures responsible for the MDR reversal activity. The most active compound was 6-hydroxy-3-(2-hydroxyethyl)-4-methyl-7-methoxycoumarin which was equally potent as the MDR modulator verapamil but failed to display toxicity in normal cells, suggesting that the presence of the 2-hydroxyethyl group is favorable for the activity [129].

Preliminary observations suggest that the activity is largely influenced by modifications of the substitution pattern, particularly by the presence of hydrophobic bulk residues in the coumarin nucleus. Coumarin derivatives may become novel MDR reversal agents given their ability to



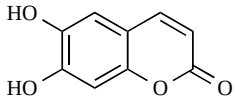
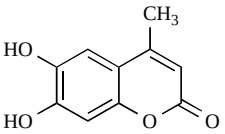
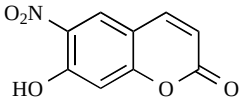
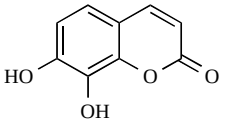
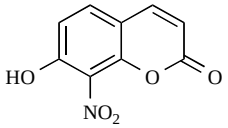
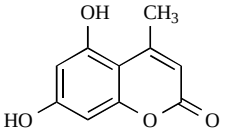
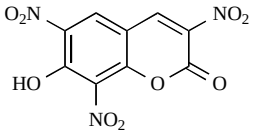
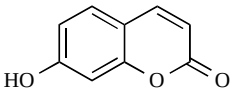
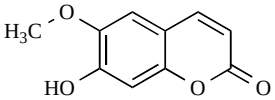
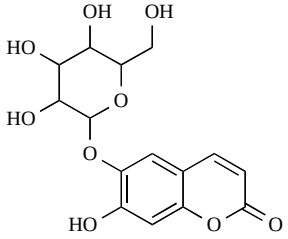
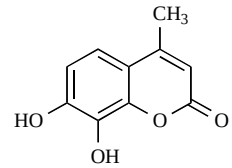
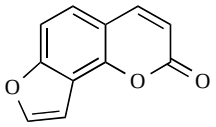
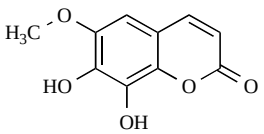
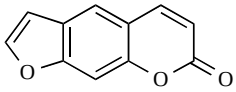
DMDCK
(±)-3'-O,4'-O-bis(3,4-dimethoxycinnamoyl)
-cis-khellactone)

Cnidiadin
2-(2-oxo-8,9-dihydro-2*H*-furo[2,3-*h*]
chromen-8-yl)propan-2-yl isobutyrate

R = 1-oxobutyl or 2-methyl-1-oxobutyl
or 3-methyl-1-oxobutyl.[206]

Fig. (5). Coumarin derivatives with multidrug resistance reversal activity.

Table 1. Simple Coumarins Mentioned in this Update

Number	Name	Structure	Number	Name	Structure
1	**Esculetin** (6,7-dihydroxy coumarin)		**8**	**4-methylesculetin** (6,7-dihydroxy-4-methylcoumarin)	
2	6-nitro-7-hydroxycoumarin		**9**	**Daphnetin** 7,8-dihydroxycoumarin	
3	8-nitro-7-hydroxycoumarin		**10**	5,7-dihydroxy-4-methylcoumarin	
4	3,6,8-trinitro-7-hydroxycoumarin		**11**	**umbelliferone** (7-hydroxy coumarin)	
5	**Scopoletin** (6-methoxy-7-hydroxycoumarin)		**12**	**Esculin** (7-hydroxy-2-oxo-2H-chromen-6-yl beta-D-glucopyranoside)	
6	**DHMC** (7,8-dihydroxy-4-methylcoumarin)		**13**	**Angelicin** furo[2,3-h]chromen-2-one	
7	**Fraxetin** (6-methoxy-7,8-dihydroxycoumarin)		**14**	**Psoralen** 7H-furo[3,2-g]chromen-7-one	

specifically inhibit Pgp in the absence of toxicity in normal cells.

DEFINING THE CLINICAL COURSE OF COUMARIN COMPOUNDS

There are several drugs in the market belonging to the coumarin family, mainly oral anticoagulants used for more than 50 years in the treatment of thromboembolic diseases [130, 131]. Other marketed coumarins include novobiocin, licensed for the treatment of human infections as supported by several clinical trials[132-134] and Venalot® Depot (Shaper & Brummer; Germany) that is used for the therapy of severe non-organic venous complaints [61].

Over two decades ago *in vivo* studies about the potential use of coumarins in cancer treatment were initiated. The treatment of patients suffering from locally advanced or metastatic renal cell carcinoma with coumarin (100 mg orally) and cimetidine induce a 6-33% of response rate (complete or partial remissions) according to the different schedules in clinical trials [135-137]. Patients showed no symptomatic organ dysfunction or toxicity. Other pilot studies were designed to evaluate the effect of coumarin and cimetidine in patients with melanoma, but unfortunately these drugs failed to exhibit any beneficial effect in this population. However a multicentre prospectively randomized double blind placebo-controlled trial showed that a daily oral dose of 50 mg coumarin prevented early recurrence of malignant melanoma. A significant reduction in the recurrence values without toxic effects associated with coumarin treatment was observed in these patients [138-140]. A multicenter trial including patients with metastatic hormone naive or hormone refractory prostatic carcinoma that received 3 g coumarin daily showed that partial responses occurred in 8% of the patients and toxicity was limited to asymptomatic hepatic transaminases elevation in three patients and nausea and vomiting in four patients [141].

In a phase I trial, a tricylic coumarin-based sulfamate (667 Coumate), that irreversibly inhibits steroid sulfatase (STS) activity was evaluated in postmenopausal women with breast cancer. Four patients showed evidence of stable disease for 2 to 7 months and decreased serum concentration of estrone, estradiol, androstenediol, and DHEA. The drug was well tolerated with only minor adverse effects [112]. It was shown that the coumarin antibiotic novobiocin potentiates the activity of etoposide (VP-16) *in vitro* by increasing intracellular accumulation of VP-16. Therefore, a clinical trial was carried out in patients with refractary cancer treated with VP-16 combined with novobiocin. Novobiocin (7 g/m^2/day) failed to augment the toxicity of VP-16 and the dose-limiting toxicities consisted of neutropenic fever and reversible hyperbilirubinemia. Nausea, which was a limiting side effect in other trials using novobiocin, was well controlled by the administration of serotonergic antiemetics. Diarrhea was common but mild in most patients [142].

In summary, most pharmacological studies involve mainly coumarin itself as an anti-neoplastic drug. In some trials, a positive outcome following coumarin treatment was observed. However, it is important to point out that treatments were generally well tolerated over a wide range of oral coumarin doses, from 50 mg to 7 g daily according to the protocol design. Self-limited side effects included insomnia, nausea, vomiting, diarrhea, and asymptomatic abnormal elevations of serum hepatic transaminases. These side effects disappeared when coumarin therapy was stopped and there was no record of significant hepatic, hematologic or renal toxicity during the trials [143].

As coumarin compounds are relatively non-toxic and they can be combined with other chemotherapeutic or biological agents to improve their efficacy, further investigations with coumarin derivatives are important to eventually develop new drugs for the treatment of cancer.

FUTURE PERSPECTIVES

In a very interesting review, Dueñas-González *et al*. arise the metaphor of drug discovery and development process as the tale *The Prince and the Pauper* by Mark Twain [144]. In accordance, we support the idea that it is not just princely, (interpreting as high cost) new drugs that can help to treat diseases that maybe that pauper (interpreting as low cost) drugs developed, could bear the same potential for efficacy. Classical drug discovery involves target discovery and validation, lead identification by high-throughput screening, and lead optimization by medicinal chemistry. Pre-clinical follow-up evaluation includes analysis in animal models of compound efficacy, pharmacology, toxicology, specificity and drug interaction studies, hence, the majority of the newer drug lead are simply cost-prohibitive by researchers at non-profit academic organizations [145]. This relevant issue led to reflect upon alternatives for drug development strategy, as named drug *repositioning, drug repurposing*, or *indication switch*. The *repositioning* term refers to the exploitation of established drugs that have already been approved for the treatment of certain diseases and expand their therapeutical indication to other human pathologies.

Based on the pharmacovigilance data of the prescribed coumarin derivatives, their presence in the diet and herbal medicines, their low toxicity against normal cells and selectively for neoplastic cells, we firmly believe that the potential of coumarin compounds as chemotherapeutic agents needs to be further investigated. Although some coumarin compounds seem to be privileged structures for at least some biological activities, there remains the challenge to design and synthesize molecules with high specific affinity for other pharmacologically important targets or to characterize their mechanism of action to become available therapeutics drugs. This review highlights the progress that has been made in the development of coumarin scaffolds for anti-cancer drug discovery.

Several molecules with a coumarin framework were reported to have multiple biological activities (Fig. **6**). These studies strongly support that the biological activity and therapeutic applications of coumarins rely on their chemical structure, namely, the pattern of substitution on the aromatic ring.

We would like to convey the concept of crosstalk from biology to the drug design process in medicinal chemistry. At least for coumarin molecules, some pharmacophoric groups can bring about several biological effects. Current

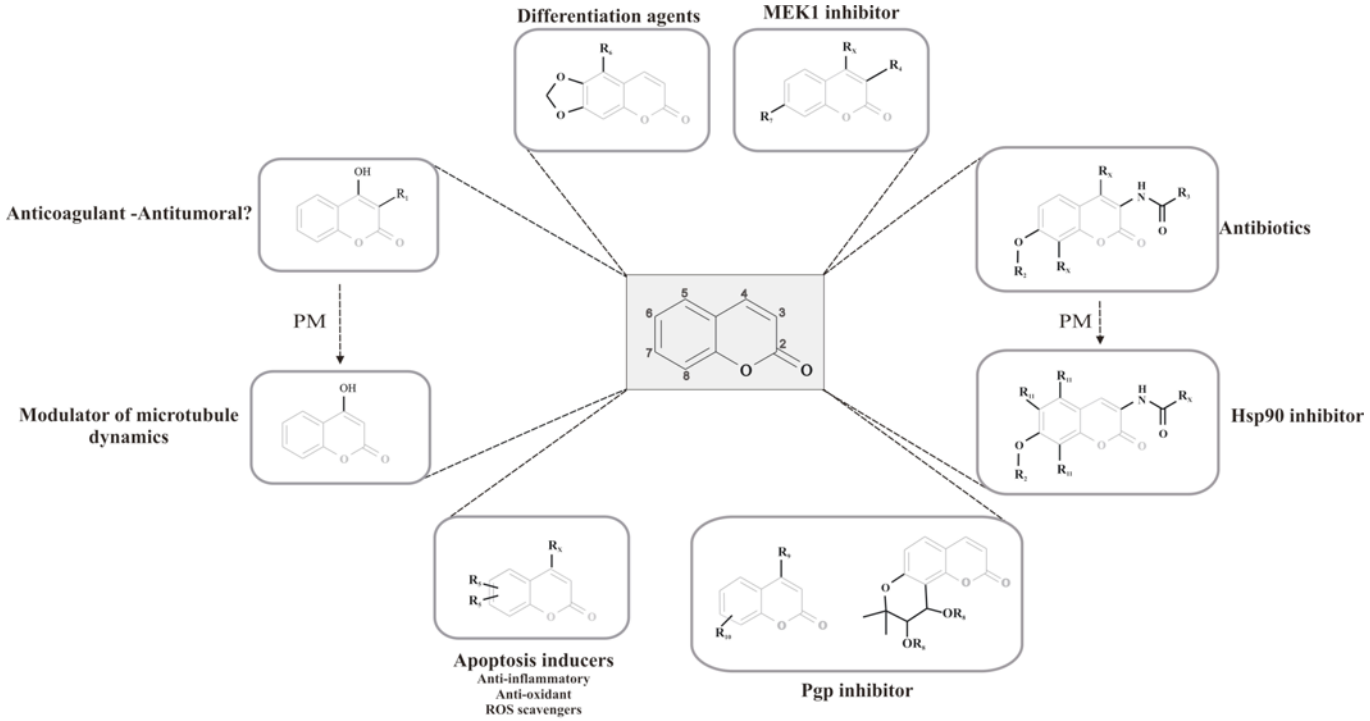


Fig. (6). Coumarin framework and lead compounds with diverse biological activities.

R_1: Carbon chain residue. R_2: H-bond donor residue (noviose moiety). R_3: Bulky residue. R_4: Hydrophobic residue. R_5: Polar groups (hidroxyl; methoxy; acetoxy). R_6: Alkoxy residue. R_7: Carbamate residue. R_8: Hydrophobic and H-bond aceptor residue. R_9: Hydrophobic bulky residue. R_{10}: H-bond donor and bulky residue. R_{11}: H-bond acceptor. R_x: Not essential group. ROS: reactive oxygen species; Pgp: P-glycoprotein; PM: Pharmacomodulation.

findings suggest that certain structural features, such as the neighboring dihydroxy functionality in simple coumarins, are not only important for their promoting ROS scavenging action but also for their anti-inflammatory and pro-apoptotic activity in cancer cells. It is clear that the redox properties of these molecules may lead to several effects *in vivo* and in some situations turn into side effects. As it was previously discussed, certain substituents at position 4 and 3 in the coumarin nucleus are structural requirements for the anticoagulant activity, so this should be considered when introducing modifications in the pyrone ring to avoid side-effects.

This comprehensive review focused on the current literature on the structure-activity relationship of coumarin derivatives. This knowledge is crucial for the understanding of their pharmacological properties, mechanism of action and potential future therapeutic applications of these compounds as anti-cancer agents. Further studies will certainly reveal new aspects of coumarins that may eventually result in the design and development of promising coumarin clinical candidates in the near future.

ACKNOWLEDGEMENTS

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REFERENCES

[1] Crum Brown, A.; Frazer, T. *Trans. Roy. Soc. Edinburgh* **1868**, *9*(25), 151.

[2] Barton, D.; Ollis, W. D. *Comprehensive organic chemistry: the synthesis and reactions of organic compounds*. 1st edition ed.; Pergamon Press: 1979; Vol. 4, p 1205

[3] Galkin, A.; Fallarero, A.; Vuorela, P. M. Coumarins permeability in Caco-2 cell model. *J. Pharm. Pharmacol.* **2009**, *61*(2), 177-84.

[4] Egan, D.; O'Kennedy, R.; Moran, E.; Cox, D.; Prosser, E.; Thornes, R. D. The pharmacology, metabolism, analysis, and applications of coumarin and coumarin-related compounds. *Drug Metab. Rev.* **1990**, *22*(5), 503-29.

[5] Born, S. L.; Api, A. M.; Ford, R. A.; Lefever, F. R.; Hawkins, D. R. Comparative metabolism and kinetics of coumarin in mice and rats. *Food Chem. Toxicol.* **2003**, *41*(2), 247-58.

[6] Ratanasavanh, D.; Lamiable, D.; Biour, M.; Guedes, Y.; Gersberg, M.; Leutenegger, E.; Riche, C. Metabolism and toxicity of coumarin on cultured human, rat, mouse and rabbit hepatocytes. *Fundam. Clin. Pharmacol.* **1996**, *10*(6), 504-10.

[7] Evans, J. G.; Gaunt, I. F.; Lake, B. G. Two-year toxicity study on coumarin in the baboon. *Food Cosmet. Toxicol.* **1979**, *17*(3), 187-93.

[8] Finn, G. J.; Kenealy, E.; Creaven, B. S.; Egan, D. A. *In vitro* cytotoxic potential and mechanism of action of selected coumarins, using human renal cell lines. *Cancer Lett.* **2002**, *183*(1), 61-8.

[9] Egan, D.; James, P.; Cooke, D.; O'Kennedy, R. Studies on the cytostatic and cytotoxic effects and mode of action of 8-nitro-7-hydroxycoumarin. *Cancer Lett.* **1997**, *118*(2), 201-11.

[10] O'Kennedy, R.; Thornes, R. D. *Coumarins: Biology, Applications and Mode of Action*. Chichester, 1997.

[11] Au, N.; Rettie, A. E. Pharmacogenomics of 4-hydroxycoumarin anticoagulants. *Drug Metab. Rev.* **2008**, *40*(2), 355-75.

[12] Oldenburg, J.; Watzka, M.; Rost, S.; Muller, C. R. VKORC1: molecular target of coumarins. *J Thromb Haemost* **2007**, *5 Suppl 1*, 1-6.

[13] Ikawa, M.; Stahmann, M.; Link, P. Studies on 4-hydroxycoumarin. *J. Am. Chem. Soc* **1944**, *66*, 902-6.
[14] Gebauer, M. Synthesis and structure-activity relationships of novel warfarin derivatives. *Bioorg. Med. Chem.* **2007**, *15*(6), 2414-20.
[15] Zacharski, L. R.; Meehan, K. R.; Algarra, S. M.; Calvo, F. A. Clinical trials with anticoagulant and antiplatelet therapies. *Cancer Metastasis Rev.* **1992**, *11*(3-4), 421-31.
[16] McCulloch, P.; George, W. D. Warfarin inhibits metastasis of Mtln3 rat mammary carcinoma without affecting primary tumour growth. *Br. J. Cancer* **1989**, *59*(2), 179-83.
[17] Velasco-Velazquez, M. A.; Agramonte-Hevia, J.; Barrera, D.; Jimenez-Orozco, A.; Garcia-Mondragon, M. J.; Mendoza-Patino, N.; Landa, A.; Mandoki, J. 4-Hydroxycoumarin disorganizes the actin cytoskeleton in B16-F10 melanoma cells but not in B82 fibroblasts, decreasing their adhesion to extracellular matrix proteins and motility. *Cancer Lett.* **2003**, *198*(2), 179-86.
[18] Velasco-Velazquez, M. A.; Salinas-Jazmin, N.; Mendoza-Patino, N.; Mandoki, J. J. Reduced paxillin expression contributes to the antimetastatic effect of 4-hydroxycoumarin on B16-F10 melanoma cells. *Cancer Cell Int* **2008**, *8*, 8.
[19] Kostova, I. Synthetic and natural coumarins as cytotoxic agents. *Curr Med Chem Anticancer Agents* **2005**, *5*(1), 29-46.
[20] Madari, H.; Panda, D.; Wilson, L.; Jacobs, R. S. Dicoumarol: a unique microtubule stabilizing natural product that is synergistic with Taxol. *Cancer Res.* **2003**, *63*(6), 1214-20.
[21] Buey, R. M.; Barasoain, I.; Jackson, E.; Meyer, A.; Giannakakou, P.; Paterson, I.; Mooberry, S.; Andreu, J. M.; Diaz, J. F. Microtubule interactions with chemically diverse stabilizing agents: thermodynamics of binding to the paclitaxel site predicts cytotoxicity. *Chem. Biol.* **2005**, *12*(12), 1269-79.
[22] Maxwell, A. The interaction between coumarin drugs and DNA gyrase. *Mol. Microbiol.* **1993**, *9*(4), 681-6.
[23] Lewis, R. J.; Singh, O. M.; Smith, C. V.; Skarzynski, T.; Maxwell, A.; Wonacott, A. J.; Wigley, D. B. The nature of inhibition of DNA gyrase by the coumarins and the cyclothialidines revealed by X-ray crystallography. *EMBO J.* **1996**, *15*(6), 1412-20.
[24] Anderle, C.; Stieger, M.; Burrell, M.; Reinelt, S.; Maxwell, A.; Page, M.; Heide, L. Biological activities of novel gyrase inhibitors of the aminocoumarin class. *Antimicrob. Agents Chemother.* **2008**, *52*(6), 1982-90.
[25] Radl, S. Structure-activity relationships in DNA gyrase inhibitors. *Pharmacol. Ther.* **1990**, *48*(1), 1-17.
[26] Li, S. M.; Heide, L. New aminocoumarin antibiotics from genetically engineered Streptomyces strains. *Curr. Med. Chem.* **2005**, *12*(4), 419-27.
[27] Laurin, P.; Ferroud, D.; Klich, M.; Dupuis-Hamelin, C.; Mauvais, P.; Lassaigne, P.; Bonnefoy, A.; Musicki, B. Synthesis and *in vitro* evaluation of novel highly potent coumarin inhibitors of gyrase B. *Bioorg. Med. Chem. Lett.* **1999**, *9*(14), 2079-84.
[28] Laurin, P.; Ferroud, D.; Schio, L.; Klich, M.; Dupuis-Hamelin, C.; Mauvais, P.; Lassaigne, P.; Bonnefoy, A.; Musicki, B. Structure-activity relationship in two series of aminoalkyl substituted coumarin inhibitors of gyrase B. *Bioorg. Med. Chem. Lett.* **1999**, *9*(19), 2875-80.
[29] Xiao, L.; Lu, X.; Ruden, D. M. Effectiveness of hsp90 inhibitors as anti-cancer drugs. *Mini Rev Med Chem* **2006**, *6*(10), 1137-43.
[30] Burlison, J. A.; Avila, C.; Vielhauer, G.; Lubbers, D. J.; Holzbeierlein, J.; Blagg, B. S. Development of novobiocin analogues that manifest anti-proliferative activity against several cancer cell lines. *J. Org. Chem.* **2008**, *73*(6), 2130-7.
[31] Donnelly, A.; Blagg, B. S. Novobiocin and additional inhibitors of the Hsp90 C-terminal nucleotide-binding pocket. *Curr. Med. Chem.* **2008**, *15*(26), 2702-17.
[32] Radanyi, C.; Le Bras, G.; Messaoudi, S.; Bouclier, C.; Peyrat, J. F.; Brion, J. D.; Marsaud, V.; Renoir, J. M.; Alami, M. Synthesis and biological activity of simplified denoviose-coumarins related to novobiocin as potent inhibitors of heat-shock protein 90(hsp90). *Bioorg. Med. Chem. Lett.* **2008**, *18*(7), 2495-8.
[33] Burlison, J. A.; Neckers, L.; Smith, A. B.; Maxwell, A.; Blagg, B. S. Novobiocin: redesigning a DNA gyrase inhibitor for selective inhibition of hsp90. *J. Am. Chem. Soc.* **2006**, *128*(48), 15529-36.
[34] Yu, X. M.; Shen, G.; Neckers, L.; Blake, H.; Holzbeierlein, J.; Cronk, B.; Blagg, B. S. Hsp90 inhibitors identified from a library of novobiocin analogues. *J. Am. Chem. Soc.* **2005**, *127*(37), 12778-9.
[35] Donnelly, A. C.; Mays, J. R.; Burlison, J. A.; Nelson, J. T.; Vielhauer, G.; Holzbeierlein, J.; Blagg, B. S. The design, synthesis, and evaluation of coumarin ring derivatives of the novobiocin scaffold that exhibit antiproliferative activity. *J. Org. Chem.* **2008**, *73*(22), 8901-20.
[36] Le Bras, G.; Radanyi, C.; Peyrat, J. F.; Brion, J. D.; Alami, M.; Marsaud, V.; Stella, B.; Renoir, J. M. New novobiocin analogues as antiproliferative agents in breast cancer cells and potential inhibitors of heat shock protein 90. *J. Med. Chem.* **2007**, *50*(24), 6189-200.
[37] Radanyi, C.; Le Bras, G.; Bouclier, C.; Messaoudi, S.; Peyrat, J. F.; Brion, J. D.; Alami, M.; Renoir, J. M. Tosylcyclonovobiocic acids promote cleavage of the hsp90-associated cochaperone p23. *Biochem. Biophys. Res. Commun.* **2009**, *379*(2), 514-8.
[38] Chu, C. Y.; Tsai, Y. Y.; Wang, C. J.; Lin, W. L.; Tseng, T. H. Induction of apoptosis by esculetin in human leukemia cells. *Eur J Pharmacol* **2001**, *416*(1-2), 25-32.
[39] Yang, J. Y.; Della-Fera, M. A.; Baile, C. A. Esculetin induces mitochondria-mediated apoptosis in 3T3-L1 adipocytes. *Apoptosis* **2006**, *11*(8), 1371-8.
[40] Kuo, H. C.; Lee, H. J.; Hu, C. C.; Shun, H. I.; Tseng, T. H. Enhancement of esculetin on Taxol-induced apoptosis in human hepatoma HepG2 cells. *Toxicol Appl Pharmacol* **2006**, *210*(1-2), 55-62.
[41] Finn, G.; Creaven, B.; Egan, D. Modulation of mitogen-activated protein kinases by 6-nitro-7-hydroxycoumarin mediates apoptosis in renal carcinoma cells. *Eur J Pharmacol* **2003**, *481*(2-3), 159-67.
[42] Finn, G. J.; Creaven, B.; Egan, D. A. Study of the *in vitro* cytotoxic potential of natural and synthetic coumarin derivatives using human normal and neoplastic skin cell lines. *Melanoma Res.* **2001**, *11*(5), 461-7.
[43] Kim, E. K.; Kwon, K. B.; Shin, B. C.; Seo, E. A.; Lee, Y. R.; Kim, J. S.; Park, J. W.; Park, B. H.; Ryu, D. G. Scopoletin induces apoptosis in human promyeloleukemic cells, accompanied by activations of nuclear factor kappaB and caspase-3. *Life Sci* **2005**, *77*(7), 824-36.
[44] Liu, X. L.; Zhang, L.; Fu, X. L.; Chen, K.; Qian, B. C. Effect of scopoletin on PC3 cell proliferation and apoptosis. *Acta Pharmacologica Sinica* **2001**, *22*, 929-933.
[45] Goel, A.; Prasad, A. K.; Parmar, V. S.; Ghosh, B.; Saini, N. 7,8-Dihydroxy-4-methylcoumarin induces apoptosis of human lung adenocarcinoma cells by ROS-independent mitochondrial pathway through partial inhibition of ERK/MAPK signaling. *FEBS Lett* **2007**, *581*(13), 2447-54.
[46] Riveiro, M. E.; Vazquez, R.; Moglioni, A.; Gomez, N.; Baldi, A.; Davio, C.; Shayo, C. Biochemical mechanisms underlying the pro-apoptotic activity of 7,8-dihydroxy-4-methylcoumarin in human leukemic cells. *Biochem Pharmacol* **2008**, *75*(3), 725-36.
[47] Kolodziej, H.; Kayser, O.; Woerdenbag, H. J.; van Uden, W.; Pras, N. Structure-cytotoxicity relationships of a series of natural and semi-synthetic simple coumarins as assessed in two human tumour cell lines. *Z Naturforsch [C]* **1997**, *52*(3-4), 240-4.
[48] Kawase, M.; Sakagami, H.; Hashimoto, K.; Tani, S.; Hauer, H.; Chatterjee, S. S. Structure-cytotoxic activity relationships of simple hydroxylated coumarins. *Anticancer Res.* **2003**, *23*(4), 3243-6.
[49] Riveiro, M. E.; Moglioni, A.; Vazquez, R.; Gomez, N.; Facorro, G.; Piehl, L.; de Celis, E. R.; Shayo, C.; Davio, C. Structural insights into hydroxycoumarin-induced apoptosis in U-937 cells. *Bioorg Med Chem* **2008**, *16*(5), 2665-75.
[50] Fylaktakidou, K. C.; Hadjipavlou-Litina, D. J.; Litinas, K. E.; Nicolaides, D. N. Natural and synthetic coumarin derivatives with anti-inflammatory/ antioxidant activities. *Curr. Pharm. Des.* **2004**, *10*(30), 3813-33.
[51] Kimura, Y.; Okuda, H.; Arichi, S.; Baba, K.; Kozawa, M. Inhibition of the formation of 5-hydroxy-6,8,11,14-eicosatetraenoic acid from arachidonic acid in polymorphonuclear leukocytes by various coumarins. *Biochim. Biophys. Acta* **1985**, *834*(2), 224-9.
[52] Neichi, T.; Koshihara, Y.; Murota, S. Inhibitory effect of esculetin on 5-lipoxygenase and leukotriene biosynthesis. *Biochim. Biophys. Acta* **1983**, *753*(1), 130-2.
[53] Zatta, A.; Bevilacqua, C. Differential inhibition of polymorphonuclear leucocyte functions by cloricromene. *Pharmacol. Res.* **1999**, *40*(6), 525-33.
[54] Malhotra, S.; Shakya, G.; Kumar, A.; Vanhoecke, B. W.; Cholli, A.

L.; Raj, H. G.; Saso, L.; Ghosh, B.; Bracke, M. E.; Prasad, A. K.; Biswal, S.; Parmar, V. S. Antioxidant, Antiinflammatory and Anti-invasive Activities of Biopolyphenolics *ARKIVOC* **2008**,*(vi)*, 119-139.

[55] Raj, H. G.; Parmar, V. S.; Jain, S. C.; Goel, S.; Poonam; Himanshu; Malhotra, S.; Singh, A.; Olsen, C. E.; Wengel, J. Mechanism of biochemical action of substituted 4-methylbenzopyran-2-ones. Part I: Dioxygenated 4-methyl coumarins as superb antioxidant and radical scavenging agents. *Bioorg. Med. Chem.* **1998**, *6*(6), 833-9.

[56] Ishihara, M.; Yokote, Y.; Sakagami, H. Quantitative structure-cytotoxicity relationship analysis of coumarin and its derivatives by semiempirical molecular orbital method. *Anticancer Res* **2006**, *26*(4B), 2883-6.

[57] Koshy, L.; Dwarakanath, B. S.; Raj, H. G.; Chandra, R.; Mathew, T. L. Suicidal oxidative stress induced by certain antioxidants. *Indian J Exp Biol* **2003**, *41*(11), 1273-8.

[58] Khan, N. S.; Ahmad, A.; Hadi, S. M. Anti-oxidant, pro-oxidant properties of tannic acid and its binding to DNA. *Chem Biol Interact Mar* **2000**, *125*(3), 177-89.

[59] Giovannini, C.; Scazzocchio, B.; Vari, R.; Santangelo, C.; D'Archivio, M.; Masella, R. Apoptosis in cancer and atherosclerosis: polyphenol activities. *Ann. Ist. Super. Sanita* **2007**, *43*(4), 406-16.

[60] Laughton, M. J.; Evans, P. J.; Moroney, M. A.; Hoult, J. R.; Halliwell, B. Inhibition of mammalian 5-lipoxygenase and cyclo-oxygenase by flavonoids and phenolic dietary additives. Relationship to antioxidant activity and to iron ion-reducing ability. *Biochem Pharmacol* **1991**, *42*(9), 1673-81.

[61] Hoult, J. R.; Paya, M. Pharmacological and biochemical actions of simple coumarins: natural products with therapeutic potential. *Gen Pharmacol* **1996**, *27*(4), 713-22.

[62] Paya, M.; Goodwin, P. A.; De Las Heras, B.; Hoult, J. R. Superoxide scavenging activity in leukocytes and absence of cellular toxicity of a series of coumarins. *Biochem Pharmacol* **1994**, *48*(3), 445-51.

[63] Ma, J.; Jones, S. H.; Hecht, S. M. A coumarin from Mallotus resinosus that mediates DNA cleavage. *J Nat Prod* **2004**, *67*(9), 1614-6.

[64] Fujisawa, S.; Atsumi, T.; Kadoma, Y.; Sakagami, H. Antioxidant and prooxidant action of eugenol-related compounds and their cytotoxicity. *Toxicology* **2002**, *177*(1), 39-54.

[65] Atsumi, T.; Tonosaki, K.; Fujisawa, S. Induction of early apoptosis and ROS-generation activity in human gingival fibroblasts(HGF) and human submandibular gland carcinoma(HSG) cells treated with curcumin. *Arch. Oral Biol.* **2006**, *51*(10), 913-21.

[66] Elbling, L.; Weiss, R. M.; Teufelhofer, O.; Uhl, M.; Knasmueller, S.; Schulte-Hermann, R.; Berger, W.; Micksche, M. Green tea extract and(-)-epigallocatechin-3-gallate, the major tea catechin, exert oxidant but lack antioxidant activities. *FASEB J.* **2005**, *19*(7), 807-9.

[67] Tinhofer, I.; Bernhard, D.; Senfter, M.; Anether, G.; Loeffler, M.; Kroemer, G.; Kofler, R.; Csordas, A.; Greil, R. Resveratrol, a tumor-suppressive compound from grapes, induces apoptosis *via* a novel mitochondrial pathway controlled by Bcl-2. *FASEB J.* **2001**, *15*(9), 1613-5.

[68] Hail, N. Jr.; Lotan, R. Cancer chemoprevention and mitochondria: targeting apoptosis in transformed cells *via* the disruption of mitochondrial bioenergetics/redox state. *Mol Nutr Food Res* **2009**, *53*(1), 49-67.

[69] Yoo, C. B.; Han, K. T.; Cho, K. S.; Ha, J.; Park, H. J.; Nam, J. H.; Kil, U. H.; Lee, K. T. Eugenol isolated from the essential oil of Eugenia caryophyllata induces a reactive oxygen species-mediated apoptosis in HL-60 human promyelocytic leukemia cells. *Cancer Lett.* **2005**, *225*(1), 41-52.

[70] Moridani, M. Y.; Galati, G.; O'Brien, P. J. Comparative quantitative structure toxicity relationships for flavonoids evaluated in isolated rat hepatocytes and HeLa tumor cells. *Chem. Biol. Interact.* **2002**, *139*(3), 251-64.

[71] Giles, G. I. The redox regulation of thiol dependent signaling pathways in cancer. *Curr. Pharm. Des.* **2006**, *12*(34), 4427-43.

[72] Lin, T. H.; Lu, F. J.; Yin, Y. F.; Tseng, T. H. Enhancement of esculetin on arsenic trioxide-provoked apoptosis in human leukemia U937 cells. *Chem Biol Interact* **2009**, *180*(1), 61-8.

[73] Finn, G. J.; Creaven, B. S.; Egan, D. A. Investigation of intracellular signalling events mediating the mechanism of action of 7-hydroxycoumarin and 6-nitro-7-hdroxycoumarin in human renal cells. *Cancer Lett.* **2004**, *205*(1), 69-79.

[74] Han, S.; Zhou, V.; Pan, S.; Liu, Y.; Hornsby, M.; McMullan, D.; Klock, H. E.; Haugen, J.; Lesley, S. A.; Gray, N.; Caldwell, J.; Gu, X. J. Identification of coumarin derivatives as a novel class of allosteric MEK1 inhibitors. *Bioorg. Med. Chem. Lett.* **2005**, *15*(24), 5467-73.

[75] Sirangelo, I.; Iannuzzi, C.; Vilasi, S.; Irace, G.; Giuberti, G.; Misso, G.; D'Alessandro, A.; Abbruzzese, A.; Caraglia, M. W7FW14F apomyoglobin amyloid aggregates-mediated apoptosis is due to oxidative stress and AKT inactivation caused by Ras and Rac. *J. Cell. Physiol.* **2009**.

[76] Park, J. H.; Kim, E. J.; Jang, H. Y.; Shim, H.; Lee, K. K.; Jo, H. J.; Kim, H. J.; Yang, S. H.; Jeong, E. T.; Kim, H. R. Combination treatment with arsenic trioxide and sulindac enhances apoptotic cell death in lung cancer cells *via* activation of oxidative stress and mitogen-activated protein kinases. *Oncol. Rep.* **2008**, *20*(2), 379-84.

[77] Yang, E. B.; Zhao, Y. N.; Zhang, K.; Mack, P. Daphnetin, one of coumarin derivatives, is a protein kinase inhibitor. *Biochem. Biophys. Res. Commun.* **1999**, *260*(3), 682-5.

[78] Wang, C. J.; Hsieh, Y. J.; Chu, C. Y.; Lin, Y. L.; Tseng, T. H. Inhibition of cell cycle progression in human leukemia HL-60 cells by esculetin. *Cancer Lett.* **2002**, *183*(2), 163-8.

[79] Lee, S. H.; Park, C.; Jin, C. Y.; Kim, G. Y.; Moon, S. K.; Hyun, J. W.; Lee, W. H.; Choi, B. T.; Kwon, T. K.; Yoo, Y. H.; Choi, Y. H. Involvement of extracellular signal-related kinase signaling in esculetin induced G1 arrest of human leukemia U937 cells. *Biomed. Pharmacother.* **2008**, *62*(10), 723-9.

[80] López-González, J. S.; Prado-Garcia, H.; Aguilar-Cazares, D.; Molina-Guarneros, J. A.; Morales-Fuentes, J.; Mandoki, J. J. Apoptosis and cell cycle disturbances induced by coumarin and 7-hydroxycoumarin on human lung carcinoma cell lines. *Lung Cancer* **2004**, *43*(3), 275-83.

[81] Kawaii, S.; Tomono, Y.; Ogawa, K.; Sugiura, M.; Yano, M.; Yoshizawa, Y. The antiproliferative effect of coumarins on several cancer cell lines. *Anticancer Res.* **2001**, *21*(2A), 917-23.

[82] Seliger, B.; Pettersson, H. 7-Hydroxycoumarin inhibits oncogene-induced transformation of murine fibroblasts. *J. Cancer Res. Clin. Oncol.* **1994**, *120 Suppl*, S23-7.

[83] Kahn, J.; Preis, P.; Waldman, F.; Tseng, A. Jr. Coumarin modulates the cell-cycle progression of an MTV-EJras cell line. *J. Cancer Res. Clin. Oncol.* **1994**, *120 Suppl*, S19-22.

[84] Gallicchio, V. S.; Hulette, B. C.; Harmon, C.; Marshall, M. E. Toxicity of coumarin(1,2-benzopyrone) on human peripheral blood mononuclear cells and human and murine bone marrow progenitor stem cells. *J. Biol. Response Mod.* **1989**, *8*(2), 116-21.

[85] Nemeikaite, A.; Cenas, N. The changes of prooxidant and antioxidant enzyme activities in bovine leukemia virus-transformed cells. Their influence on quinone cytotoxicity. *FEBS Lett.* **1993**, *326*(1-3), 65-8.

[86] Grellier, P.; Nemeikaite-Ceniene, A.; Sarlauskas, J.; Cenas, N. Role of single-electron oxidation potential and lipophilicity in the antiplasmodial *in vitro* activity of polyphenols: comparison to mammalian cells. *Z Naturforsch C* **2008**, *63*(5-6), 445-50.

[87] Kostova, I.; Manolov, I.; Momekov, G.; Tzanova, T.; Konstantinov, S.; Karaivanova, M. Cytotoxic activity of new cerium(III) complexes of bis-coumarins. *Eur J Med Chem* **2005**, *40*(12), 1246-54.

[88] Kostova, I.; Momekov, G. New zirconium(IV) complexes of coumarins with cytotoxic activity. *Eur J Med Chem* **2006**, *41*(6), 717-26.

[89] Kostova, I.; Momekov, G. New cerium(III) complexes of coumarins - synthesis, characterization and cytotoxicity evaluation. *Eur J Med Chem* **2008**, *43*(1), 178-88.

[90] Kostova, I.; Rastogi, V. K.; Kiefer, W.; Kostovski, A. New lanthanum(III) complex--synthesis, characterization, and cytotoxic activity. *Arch. Pharm.(Weinheim).* **2006**, *339*(11), 598-607.

[91] Thati, B.; Noble, A.; Creaven, B. S.; Walsh, M.; McCann, M.; Kavanagh, K.; Devereux, M.; Egan, D. A. *In vitro* anti-tumour and cyto-selective effects of coumarin-3-carboxylic acid and three of its hydroxylated derivatives, along with their silver-based complexes, using human epithelial carcinoma cell lines. *Cancer Lett.* **2007**, *248*(2), 321-31.

[92] Thati, B.; Noble, A.; Creaven, B. S.; Walsh, M.; McCann, M.;

Kavanagh, K.; Devereux, M.; Egan, D. A. A study of the role of apoptotic cell death and cell cycle events mediating the mechanism of action of 6-hydroxycoumarin-3-carboxylatosilver in human malignant hepatic cells. *Cancer Lett.* **2007**, *250*(1), 128-39.

[93] Leszczyniecka, M.; Roberts, T.; Dent, P.; Grant, S.; Fisher, P. B. Differentiation therapy of human cancer: basic science and clinical applications. *Pharmacol. Ther.* **2001**, *90*(2-3), 105-56.

[94] Nowak, D.; Stewart, D.; Koeffler, H. P. Differentiation therapy of leukemia: 3 decades of development. *Blood* **2009**, *113*(16), 3655-65.

[95] Finn, G. J.; Creaven, B. S.; Egan, D. A. Daphnetin induced differentiation of human renal carcinoma cells and its mediation by p38 mitogen-activated protein kinase. *Biochem. Pharmacol.* **2004**, *67*(9), 1779-88.

[96] Kawaii, S.; Tomono, Y.; Katase, E.; Ogawa, K.; Yano, M. Effect of coumarins on HL-60 cell differentiation. *Anticancer Res.* **2000**, *20*(4), 2505-12.

[97] Hofmanova, J.; Kozubik, A.; Dusek, L.; Pachernik, J. Inhibitors of lipoxygenase metabolism exert synergistic effects with retinoic acid on differentiation of human leukemia HL-60 cells. *Eur. J. Pharmacol.* **1998**, *350*(2-3), 273-84.

[98] Riveiro, M. E.; Shayo, C.; Monczor, F.; Fernandez, N.; Baldi, A.; De Kimpe, N.; Rossi, J.; Debenedetti, S.; Davio, C. Induction of cell differentiation in human leukemia U-937 cells by 5-oxygenated-6,7-methylenedioxycoumarins from Pterocaulon polystachyum. *Cancer Lett.* **2004**, *210*(2), 179-88.

[99] Maes, D.; Vervisch, S.; Debenedetti, S.; Davio, C.; Mangelinckx, S.; Giubellina, N.; Kimpe, N. D. Synthesis and structural revision of naturally occurring ayapin derivatives. *Tetrahedron* **2005**, *61*(9), 2505-2511.

[100] Demyttenaere, J.; Vervisch, S.; Debenedetti, S.; Coussio, J.; Maes, D.; Kimpe, N. D. Synthesis of Virgatol and Virgatenol, Two Naturally Occurring Coumarins from *Pterocaulon virgatum*(L.) DC, and 7-(2,3-Epoxy-3-methylbutoxy)-6-methoxycoumarin, Isolated from *Conyza obscura* DC. *Synthesis* **2004**, 1844-1848.

[101] Maes, D.; Riveiro, M. E.; Shayo, C.; Davio, C.; Debenedetti, S.; De Kimpe, N. Total synthesis of naturally occurring 5,6,7- and 5,7,8-trioxygenated coumarins. *Tetrahedron* **2008**, *64*, 4438-4443.

[102] Riveiro, M. E.; Maes, D.; Vázquez, R.; Vermeulen, M.; Mangelinckx, S.; Jacobs, J.; Debenedetti, S.; Shayo, C.; De Kimpe, N.; Davio, C. Towards establishing structure-activity relationships for oxygenated coumarins as differentiation inducers of promonocytic leukemic cells. *Bioorg. Med. Chem.* **2009**, *In press*.

[103] Gasparro, F. P. The role of PUVA in the treatment of psoriasis. Photobiology issues related to skin cancer incidence. *Am J Clin Dermatol* **2000**, *1*(6), 337-48.

[104] Honigsmann, H.; Tanew, A.; Brucke, J.; Ortel, B. Photosensitizing compounds in the treatment of psoriasis. *Ciba Found. Symp.* **1989**, *146*, 159-67; discussion 167-70.

[105] Lane-Brown, M. 5-Methoxy psoralen, etretinate, and UVA for psoriasis. *Int. J. Dermatol.* **1987**, *26*(10), 655-9.

[106] Day, J. M.; Purohit, A.; Tutill, H. J.; Foster, P. A.; Woo, L. W.; Potter, B. V.; Reed, M. J. The development of steroid sulfatase inhibitors for hormone-dependent cancer therapy. *Ann. N. Y. Acad. Sci.* **2009**, *1155*, 80-7.

[107] Musa, M. A.; Cooperwood, J. S.; Khan, M. O. A review of coumarin derivatives in pharmacotherapy of breast cancer. *Curr. Med. Chem.* **2008**, *15*(26), 2664-79.

[108] Purohit, A.; Woo, L. W.; Barrow, D.; Hejaz, H. A.; Nicholson, R. I.; Potter, B. V.; Reed, M. J. Non-steroidal and steroidal sulfamates: new drugs for cancer therapy. *Mol. Cell. Endocrinol.* **2001**, *171*(1-2), 129-35.

[109] Purohit, A.; Woo, L. W.; Chander, S. K.; Newman, S. P.; Ireson, C.; Ho, Y.; Grasso, A.; Leese, M. P.; Potter, B. V.; Reed, M. J. Steroid sulphatase inhibitors for breast cancer therapy. *J. Steroid Biochem. Mol. Biol.* **2003**, *86*(3-5), 423-32.

[110] Woo, L. W.; Howarth, N. M.; Purohit, A.; Hejaz, H. A.; Reed, M. J.; Potter, B. V. Steroidal and nonsteroidal sulfamates as potent inhibitors of steroid sulfatase. *J. Med. Chem.* **1998**, *41*(7), 1068-83.

[111] Stanway, S. J.; Delavault, P.; Purohit, A.; Woo, L. W.; Thurieau, C.; Potter, B. V.; Reed, M. J. Steroid sulfatase: a new target for the endocrine therapy of breast cancer. *Oncologist* **2007**, *12*(4), 370-4.

[112] Stanway, S. J.; Purohit, A.; Woo, L. W.; Sufi, S.; Vigushin, D.; Ward, R.; Wilson, R. H.; Stanczyk, F. Z.; Dobbs, N.; Kulinskaya, E.; Elliott, M.; Potter, B. V.; Reed, M. J.; Coombes, R. C. Phase I study of STX 64(667 Coumate) in breast cancer patients: the first study of a steroid sulfatase inhibitor. *Clin. Cancer Res.* **2006**, *12*(5), 1585-92.

[113] Maucher, A.; von Angerer, E. Antitumour activity of coumarin and 7-hydroxycoumarin against 7,12-dimethylbenz[a]anthracene-induced rat mammary carcinomas. *J. Cancer Res. Clin. Oncol.* **1994**, *120*(8), 502-4.

[114] von Angerer, E.; Kager, M.; Maucher, A. Antitumour activity of coumarin in prostate and mammary cancer models. *J. Cancer Res. Clin. Oncol.* **1994**, *120 Suppl*, S14-6.

[115] Omarbasha, B.; Fair, W. R.; Heston, W. D. Effect of coumarin on the normal rat prostate and on the R-3327H prostatic adenocarcinoma. *Cancer Res.* **1989**, *49*(11), 3045-9.

[116] Breier, A.; Barancik, M.; Sulova, Z.; Uhrik, B. P-glycoprotein--implications of metabolism of neoplastic cells and cancer therapy. *Curr Cancer Drug Targets* **2005**, *5*(6), 457-68.

[117] Li, X.; Li, J. P.; Yuan, H. Y.; Gao, X.; Qu, X. J.; Xu, W. F.; Tang, W. Recent advances in P-glycoprotein-mediated multidrug resistance reversal mechanisms. *Methods Find. Exp. Clin. Pharmacol.* **2007**, *29*(9), 607-17.

[118] Malhotra, S.; Bailey, D. G.; Paine, M. F.; Watkins, P. B. Seville orange juice-felodipine interaction: comparison with dilute grapefruit juice and involvement of furocoumarins. *Clin. Pharmacol. Ther.* **2001**, *69*(1), 14-23.

[119] Wang, E. J.; Casciano, C. N.; Clement, R. P.; Johnson, W. W. Inhibition of P-glycoprotein transport function by grapefruit juice psoralen. *Pharm. Res.* **2001**, *18*(4), 432-8.

[120] Wu, J. Y.; Fong, W. F.; Zhang, J. X.; Leung, C. H.; Kwong, H. L.; Yang, M. S.; Li, D.; Cheung, H. Y. Reversal of multidrug resistance in cancer cells by pyranocoumarins isolated from Radix Peucedani. *Eur. J. Pharmacol.* **2003**, *473*(1), 9-17.

[121] Fong, W. F.; Shen, X. L.; Globisch, C.; Wiese, M.; Chen, G. Y.; Zhu, G. Y.; Yu, Z. L.; Tse, A. K.; Hu, Y. J. Methoxylation of 3',4'-aromatic side chains improves P-glycoprotein inhibitory and multidrug resistance reversal activities of 7,8-pyranocoumarin against cancer cells. *Bioorg. Med. Chem.* **2008**, *16*(7), 3694-703.

[122] Shen, X.; Chen, G.; Zhu, G.; Fong, W. F.(+/-)-3'-O, 4'-O-dicynnamoyl-cis-khellactone, a derivative of(+/-)-praeruptorin A, reverses P-glycoprotein mediated multidrug resistance in cancer cells. *Bioorg. Med. Chem.* **2006**, *14*(21), 7138-45.

[123] Chang, T.; Li, J.; Sun, X.; Yu, Y.; Feng, W.; Hao, L.; Wang, Y.; Zhang, K.; Okuyama, T. Effects of Pd-Ia, a component of Chinese traditional herb Bai-Hua Qian-Hu, on Ca2+-current and action potential duration in single ventricular cells of guinea pig hearts. *Shoyakugaku Zasshi* **1993**, *47*, 279- 282.

[124] Li, J. M.; Chang, T. H.; Sun, X. D.; Hao, L. Y.; Wang, Y. P.; Yu, Y. F.; Zhang, K. Y. Effect of dl-praeruptorin A on calcium current in ventricular cells of guinea pig. *Zhongguo Yao Li Xue Bao* **1994**, *15*(6), 525-7.

[125] Raad, I.; Terreux, R.; Richomme, P.; Matera, E. L.; Dumontet, C.; Raynaud, J.; Guilet, D. Structure-activity relationship of natural and synthetic coumarins inhibiting the multidrug transporter P-glycoprotein. *Bioorg. Med. Chem.* **2006**, *14*(20), 6979-87.

[126] Raad, I.; Hay, A.; Merza, J.; Darbour, N.; Le Ray, A.; Seraphin, D.; Richomme, P.; Dumontet, C.; Guilet, D.; Dijoux-Franca, M. Evaluation of natural phenolic compounds from Clusiaceae as P-glycoprotein inhibitors. *Electronic Journal of Natural Substances* **2007**, *2*, 5-11.

[127] Bayet, C.; Fazio, C.; Darbour, N.; Berger, O.; Raad, I.; Chaboud, A.; Dumontet, C.; Guilet, D. Modulation of P-glycoprotein activity by acridones and coumarins from Citrus sinensis. *Phytother. Res.* **2007**, *21*(4), 386-90.

[128] Barthomeuf, C.; Grassi, J.; Demeule, M.; Fournier, C.; Boivin, D.; Beliveau, R. Inhibition of P-glycoprotein transport function and reversion of MDR1 multidrug resistance by cnidiadin. *Cancer Chemother. Pharmacol.* **2005**, *56*(2), 173-81.

[129] Kawase, M.; Sakagami, H.; Motohashi, N.; Hauer, H.; Chatterjee, S. S.; Spengler, G.; Vigyikanne, A. V.; Molnar, A.; Molnar, J. Coumarin derivatives with tumor-specific cytotoxicity and multidrug resistance reversal activity. *In vivo* **2005**, *19*(4), 705-11.

[130] Petersen, P.; Boysen, G.; Godtfredsen, J.; Andersen, E. D.; Andersen, B. Placebo-controlled, randomised trial of warfarin and aspirin for prevention of thromboembolic complications in chronic atrial

fibrillation. The Copenhagen AFASAK study. *Lancet* **1989**, *1*(8631), 175-9.

[131] Levine, M. N.; Hirsh, J.; Gent, M.; Turpie, A. G.; Weitz, J.; Ginsberg, J.; Geerts, W.; LeClerc, J.; Neemeh, J.; Powers, P.; *et al*. Optimal duration of oral anticoagulant therapy: a randomized trial comparing four weeks with three months of warfarin in patients with proximal deep vein thrombosis. *Thromb. Haemost.* **1995**, *74*(2), 606-11.

[132] Walsh, T. J.; Standiford, H. C.; Reboli, A. C.; John, J. F.; Mulligan, M. E.; Ribner, B. S.; Montgomerie, J. Z.; Goetz, M. B.; Mayhall, C. G.; Rimland, D.; *et al*. Randomized double-blinded trial of rifampin with either novobiocin or trimethoprim-sulfamethoxazole against methicillin-resistant *Staphylococcus aureus* colonization: prevention of antimicrobial resistance and effect of host factors on outcome. *Antimicrob. Agents Chemother.* **1993**, *37*(6), 1334-42.

[133] Raad, I.; Darouiche, R.; Hachem, R.; Sacilowski, M.; Bodey, G. P. Antibiotics and prevention of microbial colonization of catheters. *Antimicrob. Agents Chemother.* **1995**, *39*(11), 2397-400.

[134] Raad, II; Hachem, R. Y.; Abi-Said, D.; Rolston, K. V.; Whimbey, E.; Buzaid, A. C.; Legha, S. A prospective crossover randomized trial of novobiocin and rifampin prophylaxis for the prevention of intravascular catheter infections in cancer patients treated with interleukin-2. *Cancer* **1998**, *82*(2), 403-11.

[135] Dexeus, F. H.; Logothetis, C. J.; Sella, A.; Fitz, K.; Amato, R.; Reuben, J. M.; Dozier, N. Phase II study of coumarin and cimetidine in patients with metastatic renal cell carcinoma. *J. Clin. Oncol.* **1990**, *8*(2), 325-9.

[136] Kokron, O.; Maca, S.; Gasser, G.; Schmidt, P. R. Cimetidine and coumarin therapy of renal cell carcinoma. A pilot study. *Oncology* **1991**, *48*(2), 102-6.

[137] Marshall, M. E.; Mendelsohn, L.; Butler, K.; Riley, L.; Cantrell, J.; Wiseman, C.; Taylor, R.; Macdonald, J. S. Treatment of metastatic renal cell carcinoma with coumarin(1,2-benzopyrone) and cimetidine: a pilot study. *J. Clin. Oncol.* **1987**, *5*(6), 862-6.

[138] Marshall, M. E.; Butler, K.; Cantrell, J.; Wiseman, C.; Mendelsohn, L. Treatment of advanced malignant melanoma with coumarin and cimetidine: a pilot study. *Cancer Chemother. Pharmacol.* **1989**, *24*(1), 65-6.

[139] Nolte, H.; Pedersen, L.; Mouridsen, H. T. Combined treatment of advanced malignant melanoma with coumarin and cimetidine. *Anticancer Res.* **1987**, *7*(3 Pt B), 449-50.

[140] Thornes, D.; Daly, L.; Lynch, G.; Browne, H.; Tanner, A.; Keane, F.; O'Loughlin, S.; Corrigan, T.; Daly, P.; Edwards, G.; *et al*. Prevention of early recurrence of high risk malignant melanoma by coumarin. Irish Melanoma Group. *Eur. J. Surg. Oncol.* **1989**, *15*(5), 431-5.

[141] Mohler, J. L.; Gomella, L. G.; Crawford, E. D.; Glode, L. M.; Zippe, C. D.; Fair, W. R.; Marshall, M. E. Phase II evaluation of coumarin(1,2-benzopyrone) in metastatic prostatic carcinoma. *Prostate* **1992**, *20*(2), 123-31.

[142] Murren, J. R.; DiStasio, S. A.; Lorico, A.; McKeon, A.; Zuhowski, E. G.; Egorin, M. J.; Sartorelli, A. C.; Rappa, G. Phase I and pharmacokinetic study of novobiocin in combination with VP-16 in patients with refractory malignancies. *Cancer J* **2000**, *6*(4), 256-65.

[143] Cox, D.; O'Kennedy, R.; Thornes, R. D. The rarity of liver toxicity in patients treated with coumarin(1,2-benzopyrone). *Hum. Toxicol.* **1989**, *8*(6), 501-6.

[144] Duenas-Gonzalez, A.; Garcia-Lopez, P.; Herrera, L. A.; Medina-Franco, J. L.; Gonzalez-Fierro, A.; Candelaria, M. The prince and the pauper. A tale of anticancer targeted agents. *Mol Cancer* **2008**, *7*, 82.

[145] Verkman, A. S. Drug discovery in academia. *Am J Physiol Cell Physiol* **2004**, *286*(3), C465-74.