Natural and Synthetic Coumarins and their Pharmacological Activity

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ABSTRACT
Coumarins are a structurally diverse group of natural substances derived from plants that display a host of bioactivities. In this paper, we will introduce the reader to coumarins and their applications as medicinal substances. The great diversity in coumarin structure will be discussed along with their extensive use as pharmaceutical agents. Coumarins display a wide range of antimicrobial activity and applications of coumarins as antifungal and antiviral agents will be addressed. Other properties of coumarins such as their role in neuroprotection, anticancer, and as antioxidants will also be reviewed.

Keywords: coumarins, antimicrobial agents, neuroprotection, natural products in medicine

Introduction
Coumarins form an extensive group of natural substances known as secondary metabolites. They are found in over 150 different species of plants belonging to almost 30 different families. The families containing the highest content of coumarins are: Rutaceae, Clusiaceae, Guttiferae, Caprifoliaceae, Oleaceae, Nyctaginaceae and Apioaceae. Coumarin compounds accumulate in large quantities in fruits (such as citrus fruits), vegetables (e.g., celery), roots, flowers and leaves. In smaller quantities they are isolated from bark and stems.

Coumarins, in addition to occurring in vascular plants, are also found in bacteria and fungi such as novobiocin and coumermicin which are known antibiotics synthesized by bacteria. In contrast, Aspergillus flavus is a source of aflatoxin, a highly carcinogenic substance with coumarin ring in its structure.

Structural diversity of coumarins
The structural diversity of natural coumarins is the basis for classifying them into four groups:
1. coumarin derivatives, e.g., simple coumarin, compounds formed by two rings: benzene and α-piuron. Substituents are often hydroxyl, methoxy and aliphatic groups, at the C7, C6 and C3 positions of benzopyrone (Fig. 1).

Figure 1. Chemical structure of coumarin

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2. **isocoumarin derivatives**, formed by benzene rings and α-isopirone. They have substituents in positions C3, C6, C7 and C8 (Fig. 2). They are isolated mainly from fungi: *Artemisia, Aspergillus, Fusarium, Penicillium, Streptomycetes* and the few plants belonging to families: *Compositae, Leguminoseae* and *Myriaceae.*

![Figure 2. Chemical structure of isocoumarin](image)

3. **furanocoumarin derivatives**, (Fig. 3) formed by the coupling of the coumarin ring with the furan ring at the C6-C7 position (psoralen type, Fig. 3A) or in the C7-C8 position (angelicin type, Fig. 3B).

![Figure 3. Chemical structure of furanocoumarin](image)

**Figure 3A. Psoralentype**

**Figure 3B. Angelicin-type**

4. **pyrancoumarin derivatives**, coumarine ring is condensed with pyran ring (Fig. 4). Ring condensation at the C6-C7 position is defined by the xanthyletin-type (Fig. 4A), or in position C7-C8 a seselin-type (Fig. 4B).

![Figure 4. Chemical structure of pyrancoumarin](image)

**Figure 4A. Xanthyletin-type**

**Figure 4B. Seselin-type**

**Pharmacological Activity of Coumarins**

Coumarins are a group of biologically active compounds. They are produced by living organisms (plants, fungi and bacteria) as secondary metabolites. Their activities are, among others, anti-inflammatory, antithrombotic, antimicrobial, antifungal, antiviral (including anti-HIV), anticonvulsant, antioxidant, and antitumor.

**Anti-inflammatory activity of coumarins**

Coumarins (1,2-benzopyrone) have anti-inflammatory properties and have been used to treat oedema, helping wound healing. This removes protein and oedema fluid from injured tissue by stimulating phagocytosis and proteolytic enzyme production. Esculetin exhibited anti-inflammatory activity in rat colitis. Also, esculetin inhibits the cyclooxygenase and lipoxygenase enzymes, which results in an anti-inflammatory effect.

**Anticoagulant activity of coumarins**

Vitamin K is a co-catalyst for the carboxylation reaction of the glutamic acid residue with γ-carboxyglutamic acid. The carboxylation process affects the further normal activity of coagulation factors II, VII, IX and X. Warfarin interferes with the cycle of vitamin K metabolism, resulting in liver deposition of partially carboxylated and decarboxylated proteins. These proteins are characterized
by decreased procoagulant activity.\textsuperscript{4} Coumarins interfere with the carboxylation process of C and S protein, causing a procoagulant effect.\textsuperscript{1}

It has been shown that the warfarin - coumarin derivative, used as an oral anticoagulant, negatively affects the $\gamma$-carboxylation of glutamate residues of bone proteins. As a result of its action in pregnant mothers and those taking warfarin preparation, the fetal skeleton develops abnormally.\textsuperscript{6}

Warfarin has shown particularly promising results in the treatment of SCCL (Small Cell Carcinoma Lung) a tumour cell type that is characterised by a coagulation-associated pathway.\textsuperscript{9}

### Antimicrobial activity of coumarins

Most coumarins have very low antimicrobial activity, but compounds having long chain hydrocarbon substitutions such as amsinosinol and ostruthin have a high activity towards gram(+) bacteria and show antimicrobial activity on Bacillus megaterium, Micrococcus luteus, Micrococcus lysodeikticus and Staphylococcus aureus.\textsuperscript{7}

Antigenol, a coumarin derivative isolated from green fruits Aegle marmelos (L.), exhibits antimicrobial activity against bacteria of the genus Enterococcus.\textsuperscript{1} Imperatorin shows high activity towards Shigella dysenteriae.\textsuperscript{8}

Pyranocoumarins such as grandivittin, agastylin, aegelinol benzoate and osthole isolated from the root Ferula lagopamposteris (Besser) Grecescu (Apiaceae) show activity towards both gram(+) and gram(-) bacteria, for example on Staphylococcus aureus, Salmonella typhi, Enterobacter cloacae, Enterobacter aerogenes and Helicobacter pylori.\textsuperscript{10}

Coumarins are mainly isolated from higher plants, but some of them have been discovered in microorganisms. Examples include novobiocin, coumerycin, and chartreusin. Novobiocin, a secondary metabolite of Streptomyces niveusand Streptomyces spheroides exhibit very high activity towards gram(+) organisms such as Corinebacterium diphtheria, Staphylococcus aureus, Streptomyces pneumoniae, Streptomyces pyogenes and gram(-) organisms such as Haemophilus influenzae, Neisseria meningitides and Pasteurella. Coumerycin, structural similar to novobiocin, exhibits almost 50 times more potency against bacteria belonging to Escherichia coli strains and Staphylococcus aureus strains. Alsochartreusin, isolated from Streptomyces chartreusis, shows activity towards gram(+) bacteria, but due to its toxicity, chartreusin has not been tried for treatment.\textsuperscript{7}

Antituberculous activity against Mycobacterium tuberculosis was shown in inoksopeitin, umbeliferone, phellodolenol A, marmezin and xanthyletin.\textsuperscript{12}

### Antifungal activity of coumarins

The broad spectrum of antifungal activity is shown by osthol, a derivative of coumarin isolated from celery plants. This derivative demonstrates activity against Rhizoctonia solani [Kühn], Phytophthoracapsici [Leonian], Botry-
Neuroprotective activity of coumarins

Alzheimer's disease (AD) is a degenerative and progressive neurological disorder. It is characterized by variable levels of cholinergic enzymes and the formation of senile plaques containing β-amyloid proteins in cerebral tissue. In patients with Alzheimer's disease is observed decreased or unchanging levels of acetylcholinesterase (AChE), level of second enzyme butyrylcholinesterase (BChE) increase. Therefore, the levels of AChE and BChE enzymes are considered to be crucial in the treatment of this disease. Orhan et al. have demonstrated significant inhibition of acetyl- and butyrylcholinesterase levels after application with bergapten, xanthotoxin, scopoletin, umbelliferone, and 4-methylumbelliferone.

Recent computer techniques have allowed the design of an amine-substituted coumarin derivative. The synthesized compound 3-(4-[(benzyl(ethyl)amino)methyl]phenyl)-7-[4-(diethylamino)butoxy]-2H-chromen-2-one exhibits neuroprotective activity, expressed in AChE inhibition, and is a potential candidate for Alzheimer's disease treatment.

Osthole present among others in Cnidiummonnieri (L.) fruits is a commonly used substance in traditional Chinese medicine. Chen et al. investigated the effect of osthole on the demyelination process in the central nervous system of mice in an experimental model of multiple sclerosis. The results showed that osthole delayed disease progression and could find use in the treatment of multiple sclerosis.

The use of coumarins in the treatment of skin diseases and of the hematopoietic system diseases

Therapeutic use has been found for two furanocoumarin derivatives 5-MOP (5-methoxypsoralen) as an N-acetyltransferaseinhibitor and 8-MOP (8-methoxypsoralen) for phototherapy for psoriasis and vitiligo.

In treatment of the skin disorders vitiligo, psoriasis and atopic inflammation, bergapten has also been successful. Human keratinocytes of the NCTC-2544 line were exposed to bergapten and xanthotoxin and exposed to UVA light, which resulted in cell cycle inhibition in G1 phase and increase in cellular apoptosis level.

Very effective psoriasis treatment was achieved using xanthotoxin and the PUVA method which involves administering xanthotoxin gel directly onto the patient's skin and then irradiating with UVB light.

The Jurkat cell line (T-cell leukemia line) and normal lymphocytes were exposed to 8-MOP and then exposed to UVA light. There was a marked induction of apoptosis and a significant increase in caspases: 8 and 9 (initiator caspases) and 3 and 7 (effector caspases).

This method, called photophoresis, which uses extracorporeal irradiation of blood cells previously exposed to 8-MOP, has been implicated in therapy for autoimmune diseases, such as T-cell lymphoma. Photophoresis increases apoptosis in lymphocytes, causing them to die and induce the formation of postapoptotic vesicles with anti-inflammatory properties. Another feature of xanthotoxin used in vitiligo treatment is the ability to induce skin repigment. Coumarin increases the intracellular concentration of calcium ions and affects the organization of actin fibers in the cytoskeleton of melanocytes, which in turn leads to their migration.

Anticancer activity of coumarins

In research on GLC1 (small cell lung carcinoma) and COLO 320 (colorectal cancer) cell lines, it has been shown that the cytotoxicity of coumarin is due to the presence of at least two phenolic groups at the 6,7- or 6,8-position in the ring of the molecule. The proliferation of the 786-O and A-498 (kidney cancer) and DU145 and LNCaP (prostate cancer) cells line were inhibited by coumarin and its hydroxyl derivative, umbelliferone.

Several hydroxylated and methoxylated coumarin derivatives were tested for their relative cytotoxicity on four human HSC-2 tumor cell lines, HSC-3 (oral squamous cell carcinoma), A-375 (melanoma) and HL-60 (promyelocytic leukemia). It has been shown that the cytotoxicity of 6,7-dihydroxycoumarin towards HL-60 tumor cells can be further enhanced by substituting the -OH in 3 and/or position 4. Similar conclusions were made by Budzisz et al. by QSAR regression analysis of the relationship between biological activity and physicochemical properties of test compounds. The cytotoxic effect increases with increasing hydrophobic substituents in 2, 3 and 4 positions of the benzopyrene ring.

The cytotoxic activity of organometallic coumarin complexes (umbelliferone, mendixan, warfarin, coumachlor, and niffcoumar) towards P3HR1, K-562 and THP-1 leukemia cells lines were confirmed. Nitrocoumarin derivatives of 7-hydroxy-6-nitrocoumarin and 7-hydroxy-3,6,8-trinitrocoumarin exhibited cytotoxic activity against tumor cells of the melanocytic line (SK-MEL-31). On the other hand, 8-nitro-7-hydroxy-coumarin induced apoptosis of leukemic cell lines K562 and HL-60.

Coumarin derivatives exhibit specific cytotoxicity, which is closely related to the chemical structure of their molecules. Attemptshave been made to synthesize a coumarin-like compound with selective and targeted action on tumor cells. Extremely cytotoxic heterocyclic coumarin derivatives which have 1,2,4-triazole, 4,5-dicyanoimidazole or purine groups have been obtained. In addition, the 1,2,4-triazole-3-carboxamide derivative exhibited particular selectivity to HeLa human epithelial
It has been observed that combination therapy with dicumarol, coupled with a chemotherapeutic agent, can improve efficacy and reduce toxicity compared to coumarin alone. The use of the dicumarol with taxol complex has antiproliferative effects on the hedgehog larvae (Strongylocentrotuspurpuratus) [Stimpson]. The positive result was explained by the synergism of the cytostatic and dicumarol. The authors suggest that the future of the development of combined pharmacotherapy may be the basis of modern chemotherapy.

It has been found that coumarins eaten in human diet can positively affect the body. Observations indicate that even if present at low levels in apiaceous vegetables, imperatorin, trioxalen and isopimpinellin may contribute significantly to CYP1A2 inhibition and potentially decreased procarcinogen activation.

**Conclusion**

Coumarins are a large group of biologically active compounds commonly used in natural medicine.

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