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Review Article:

# Synthesis and Pharmacological Profiles of 6,7-Dihydroxycoumarin and Its Derivatives: A Concise Review

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**Abstract**

**Background**: Esculetin, scientifically referred to as 6,7-dihydroxycoumarin, functions as the primary bioactive constituent found in *Cortex Fraxini* (commonly known as ash bark), an ancient Asian medicinal substance. Herbal practitioners utilize the outer layer of the branch or stem bark of *Cortex Fraxini* for its gentle and safe medicinal properties and its potential as a nutritional component. In contemporary times, the landscape has undergone a notable transformation due to the emergence of a wide range of innovative 6,7-dihydroxycoumarin derivatives. The recent surge of innovation has sparked a heightened interest in understanding the molecular mechanisms that underlie the effects of *Cortex Fraxini* and 6,7-dihydroxycoumarin in clinical applications. **Aim:** This succinct review seeks to build up the extensive knowledge accumulated in the past decade concerning the synthesis, pharmacological profiles and principles linked to 6,7-dihydroxycoumarin and its chemical analogues. Furthermore, we aim to provide a concise yet inclusive overview of the unique characteristics of 6,7-dihydroxycoumarin. **Conclusion**: Satisfying these aims can enhance the comprehension of the diverse possibilities presented by this chemical and its related compounds across different research and application domains.



## 1. Introduction

Since ancient times, Asian herbal medicine has effectively treated various illnesses. One of the Asian herbal medicines (AHM) that is most frequently employed is known as ash bark. *Fraxinus chinensis Roxb* (common name: Chinese ash), *Fraxinus rhynchop hylla Hance* (common name: Manchurian ash), *Fraxinus stylosa Lingelsh* (common name: Styloza ash), and *Fraxinus aboana Lingelsh* are the four species of ash bark, also called *Cortex Fraxini*, that have been assigned a classification under the AHM division and categorization system (1). It has been demonstrated that *Cortex Fraxini* displays a wide range of biological functions, some of which include anti-infective (2), inflammatory suppressant (3), painkilling (4), cancer-fighting (5), free radical scavenging (6), neuro-shielding (7), and circulatory protection (8) properties. *Cortex Fraxini* is loaded with a wide variety of useful components. It has been shown that among all the elements that make up *Cortex Fraxini*, 6,7 dihydroxy-coumarin (6,7-DHC), O-glycoside derivatives of esculetin (esculin),7-hydroxy-6-methoxycoumarin-glycoside derivatives (fraxin), and 7,8 dihydroxy-6-methoxy coumarin (fraxetin) are the most significant biologically active components, as illustrated in **Figure 1** (9).

As one of the key bioactive constituents of *Cortex Fraxini*, 6,7-DHC has been widely employed not solely for its cough relief characteristics (10) but also as an inflammatory suppressant, radical quenching agent, antimicrobial, and anticancer agents (11,12). Owing to its numerous pharmacological properties and changeable framework, 6,7-DHC has shown great potential as a promising starting point for medicinal chemists generating novel therapeutic candidates. 6,7-dihydroxyl and 3,4-unsaturation are the important chemical modification reaction points (13). This creates new 6,7-DHC derivatives that can be subjected to biological investigation. In this concise overview, we highlighted the current investigations on 6,7-DHC synthesis, pharmacological activity, mechanisms, and related substances throughout the last few years (14).

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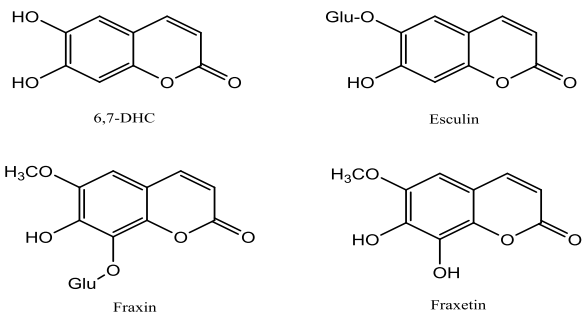
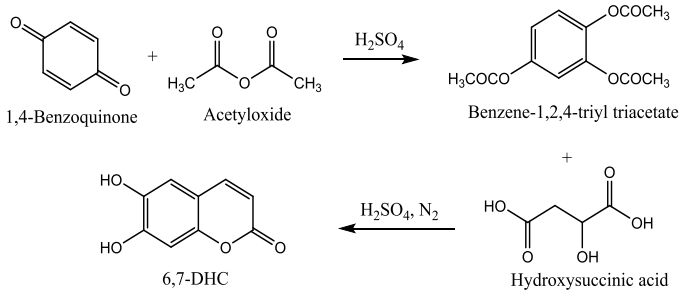



**Figure 1**. Chemical structures of coumarin derivatives extracted from *Cortex Fraxini*

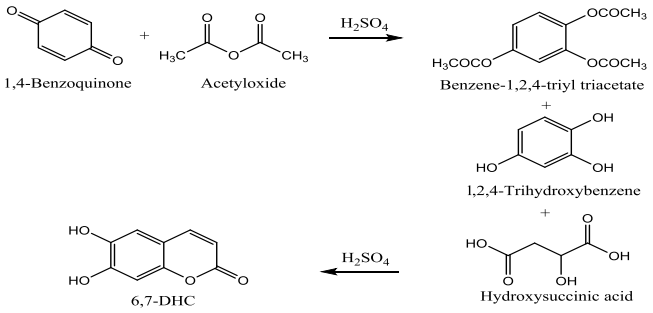
## 2. Synthesis of 6,7-DHC

In their study, Cao and his colleagues (15) explored the process of creating 6,7-DHC. They used 1,4-benzoquinone, acetyloxide, and $H_2SO_4$ as the main ingredients to produce an intermediate benzene-1,2,4-triyl triacetate. This was then mixed with strong $H_2SO_4$ and hydroxysuccinic acid to make 6,7-DHC. The total yield of the production was approximately 80% (**Scheme 1**).



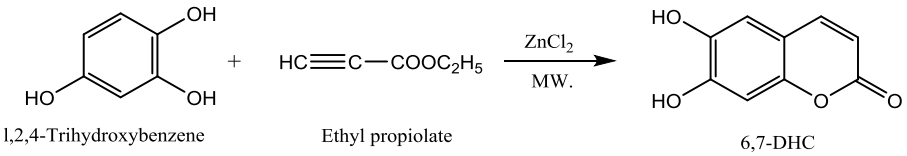

**Scheme 1**. Synthesis of 6,7-DHC

In another study (16), scientists developed an alternative synthesis pathway and enhanced the reaction parameters for optimal outcomes by utilizing singular and composite variable orthogonal examinations. The ideal reaction conditions involve a specific ratio of 1,4-benzoquinone, acetyloxide, and concentrated $H_2SO_4$, which is 1:3:0.15, respectively. This reaction should be carried out under moderate heating at 45 degrees Celsius with continuous stirring for 3 hours. Subsequently, the intermediate benzene-1,2,4-triyl triacetate was combined with an equivalent amount of 1,2,4-trihydroxybenzene under the influence of concentrated $H_2SO_4$ and hydroxysuccinic acid as catalysts. This procedure makes it possible to achieve a yield of approximately 80% of 6,7-DHC (**Scheme 2**).




**Scheme 2**. Synthesis of 6,7-DHC

Yang and colleagues (17) employed a novel methodology to synthesize 6,7-DHC, wherein the compound was produced through microwave-assisted cyclization of 1,2,4-trihydroxybenzene and ethyl propiolate. The technique utilized the catalytic effect of $ZnCl_2$. The optimal reaction conditions were carefully determined as follows: a precise ratio of 1.0:1.0 between the quantities of 1,2,4-trihydroxybenzene, and ethyl propiolate was maintained, 3.5 grams of $ZnCl_2$ were utilized, and the reaction was subjected to a 10-minute exposure at a temperature of 105°C while being subjected to a 400-watt microwave intensity. The proficient organization of components resulted in a noteworthy 87.4% production of 6,7-DHC, as illustrated in **Scheme 3** (18).

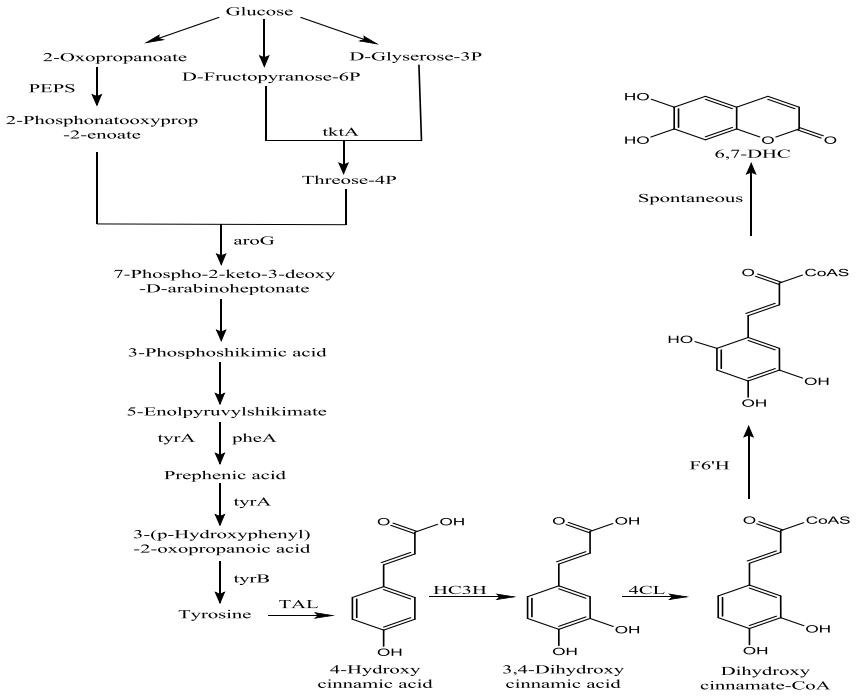



**Scheme3**. Microwave-assisted synthesis of 6,7-DHC

The researchers Yang *et al.* (19) conducted a study emphasizing the biological production of 6,7-DHC in *E. coli*. This process begins with glucose and involves a complex sequence of intermediate operations. The process commences with converting glucose into 2-oxopropanoate, D-fructopyranose-6P, and D-glyserose-3P. The enzymatic catalyst known as phospho(enol)pyruvic acid synthase (PEPS) mediates the conversion of 2-oxopropanoate to 2-phosphonatooxyprop-2-enoate. The tktA, which acts as a transketolizing enzyme, facilitates the conversion of D-fructopyranose-6P and D-glyserose-3P into threose-4P. The results mentioned above ultimately converge to form 7-phospho-2-keto-3-deoxy-D-arabinoheptonate, under the catalytic influence of the enzyme 2-dehydro-3-deoxyphosphoheptonate aldolase, also known as AroG (20).

As a result, the synthesis of 3-phosphoshikimic acid and 5-enolpyruvylshikimate occurs. The formation of prephenic acid occurs by the union of 5-enolpyruvylshikimate with prephenic acid dehydrate (pheA) and prepheic acid dehydrogenase (tyrA), resulting in the production of 4-(2-carboxy-2-oxoethyl)-4*H*-pyran-4-carboxylic acid (prephenic acid). Following this, the enzymatic action of tyrA leads to the conversion of prephenic acid into 3-(p-hydroxyphenyl)-2-oxopropanoic acid (21). Subsequently, with the aid of tyrB, which is the phenylalanine aminotransferase enzyme, Tyrosine is synthesized. The tyrosine ammonia-lyase (TAL) enzyme is of great significance in facilitating the chemical transformation of Tyrosine into 4-Hydroxycinnamic acid (22).

The enzymatic reaction involving hydroxycinnamate-3-hydroxylase (HC3H) enables the conversion of 4-hydroxycinnamic acid to 3,4-dihydroxycinnamic acid. This conversion is achieved by the cooperative action of the 4-cinnamoyl-CoA (4CL) enzyme, producing dihydroxycinnamate-CoA. The participation of the bacterial enzyme F6'H facilitates the conversion of dihydroxycinnamate-CoA into an intermediate compound. This process ultimately culminates in forming the end-product 6,7-DHC, as seen in **Scheme 4** (23).




**Scheme 4**. Synthesis of 6,7-DHC from glucose

## 3. Pharmacological profiles of 6,7-DHC

### 3.1. Inflammatory suppressant activity

The inflammatory suppressant effects of 6,7-DHC have been the subject of a significant number of molecular investigations. One of its processes includes the inhibition of the emission of nitric oxide (NO),

which Plays a part in regulating cardiac and blood vessel functions and in the attenuation of organ tissue damage brought on by inflammation. In addition, 6,7-DHC inhibits the production of protein fragments released into the bloodstream that aid cell interactions and are linked to inflammation and immune responses, leading to lesser adherence between white blood cells (WBC) and blood vessel lining cells, thereby dampening the inflammatory response (24).

In addition, it has been demonstrated that 6,7-DHC has a significant impact on reducing the severity of issues associated with inflammation. For example, it can significantly reduce the level of expression of collagenase-1 in cartilaginous tissue and lower levels of NO and eicosanoid (PGE2) in synovia, thereby delaying the onset of joint degenerative diseases (25). Moreover, 6,7-DHC demonstrated its capacity to protect the tissue in the heart from damage caused by ischemic-reoxygenation damage. It was able to accomplish this by lowering the number of inflammatory reactions that occurred throughout the body (26).

In addition, 6,7-DHC has anti-inflammatory characteristics by blocking the generation of inflammation-inducing signalling molecules during contact between fat cells and phagocytic cells (27). These interactions can occur when fat cells and lymphocytes encounter one another. The effect of this interaction can be measured by looking at how much heme oxygenase-1 (HO-1) is expressed in the body. Due to the firmly established link between obesity and the enduring low-level inflammation of lipid-rich tissue, 6,7-DHC displays promise in addressing persistent inflammation associated with obeseness (28).

### 3.2. Clot preventing activity

The prospective pharmaceutical application of 6,7-DHC in stroke management is promising, given its status as a natural derivative of coumarins like fraxetin, and umbelliferone (29). Additionally, the clinical utilization of warfarin as an anticoagulant further supports the potential efficacy of 6,7-DHC in this context (30). The excessive proliferation of vascular myocytes induced by endothelial layer impairment is a crucial contributing element to vascular proliferation-related ailments such as arterial sclerosis and recurrence of stenosis (31). 6,7-DHC has exhibited significant effectiveness in inhibiting vascular myocyte proliferation in laboratory settings, showing sensitivity to both the amount of the compound administered and the length of time it is applied (32). The elucidation of the underlying mechanism responsible for the anti-proliferative impact of 6,7-DHC has revealed that it hinders the triggering of the MAPK/ERK and phosphoinositide 3-kinase/Akt (Protein Kinase B) signalling pathways in rat sarcoma (33).

It is worth mentioning that the impact of 6,7-DHC on cell proliferation is mediated by its regulation of both the initiating factors and subsequent events within the cellular signalling cascade. This encompasses the activation of a mitogen-activated kinase cascade, the activation of phosphoinositide 3-kinase, and the initial stages of gene expression, as well as its influence on the stimulation of nuclear factor-kB and activator protein 1 transcription factor (34).

Furthermore, the efficacy of 6,7-DHC in reducing endothelial layer hyperproliferation has been established in rat models of vascular damage, indicating its potential as a therapeutic intervention for reocclusion following arterial injury (35). A recent investigation has revealed that 6,7-DHC possesses the capability to fire up the peroxisome proliferator-activated receptor gamma PPAR-γ, thus promoting the expression of ATP-binding cassette transporter (ABCA1 and G1). The choreographed sequence described later functions to impede the development of foamy macrophages originating from smooth muscles (36).

Furthermore, 6,7-DHC has demonstrated apoptosis-suppressing activities in lining cells of microvessels in mouse brains, in addition to its impact on cellular proliferation. This is supported by the observed increase in B-cell lymphoma 2 (Bcl2) expression and a simultaneous decrease in Bcl-2-associated X protein (Bax) expression, the balance between them is crucial for cell health. These effects ultimately result in the acquisition of neuroprotective properties by 6,7-DHC against brain ischemic attack/recirculation trauma. This has been confirmed through experimental studies utilizing a mouse model of middle brain arterial blockage (37,38).

### 3.3. Radical quenching activity

Oxidative radicals, characterized as chemical radicals and oxygen-containing reactive entities, naturally emerge within the organism as byproducts of oxygen metabolism. They also result from disease processes and the action of xenobiotics (39–41). In terms of cellular signalling and maintaining cellular balance, appropriate levels of oxidative radicals are crucial. Conversely, significantly elevated levels have been linked to extensive cellular structural harm. This collective scenario is termed oxidative stress (42).

As documented, it has been shown that 6,7-DHC demonstrates a robust ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, which is a chemical compound often used in antioxidant assays to examine the radical quenching activity of various substances. Furthermore, there is a notable positive association between the capacity to quench oxidative radicals and the concentration of 6,7-DHC, as well as the duration of exposure (43,44). 6,7-DHC has been identified as a potent agent in the protection of cells against oxidative radical-mediated amyloid-beta (Abeta) proteins, which demonstrates the potential of 6,7-DHC in managing the progression of Alzheimer's disease and related pathologies (45). In a separate investigation, it was found that 6,7-DHC demonstrates efficacy in guarding cells from DNA damage caused by oxidative stress (46).

### 3.4. Hepatoprotective activity

In the setting of liver disorders, the elevation of oxidative radicals and lipid peroxidation can stimulate the synthesis of the noncellular matrix. This process is marked by the heightened multiplication of hepatic perisinusoidal cells and the rapid deterioration of hepatic tissue (47). In hepatocytes, microsomal monooxygenase could metabolize 1,1-dimethylethyl hydroperoxide (also known as t-BHP), a lipid peroxides analogous substance with a shortened chain. This metabolic process can result in the formation of oxidative radical intermediates. Consequently, these radicals have the potential to induce lipid oxidative damage, thereby impacting cell integrity and ultimately causing cellular damage (48). The interaction between functional and stromal cells is a significant element in liver injury, with oxidative radicals playing a crucial role in the upregulation of thrombocyte-produced growth factor protein during the process of hepatic fibrous tissue formation (49).

Cichorium intybus and Bougainvllra spectabillis, which contain naturally occurring 6,7-DHC, have been historically utilized in customary AHM to cure different hepatic ailments (50). The extended utilization of 6,7-DHC demonstrates a favourable outcome and provides evidence of its hepatoprotective effect based on animal studies (51). The tissue histological assessment demonstrated that 6,7-DHC exhibited a mitigating effect on oxidative stress in a mouse hepatic lesion model caused by t-BHP. This effect was observed by diminished swelling of liver cells, lowered infiltration of leukocytes, and attenuation of tissue necrobiosis (52). Therefore, 6,7-DHC may have a preventive effect by interacting with radical-generating substances to mitigate hepatocyte toxicity (53,54).

### 3.5. Antihyperglycemic activity

Problems with insulin secretion or activity are at the root of diabetes, a metabolic condition that causes abnormalities in blood glucose levels. Reduced antioxidant enzyme activity is a common consequence of this condition. 6,7-DHC has been utilized to reduce the effects of oxidative stress (55,56). The administration of 6,7-DHC at a dosage of 40 mg/kg was seen to result in a reduction of blood sugar levels and a rise in serum insulin levels in male rats with diabetes induced by streptozotocin (STZ). Furthermore, the intervention resulted in the reinstatement of the level of oxidative stress defence enzymes in liver and kidney tissues, specifically glutathione S-transferase (GST), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), thiobarbituric acid reactive substances (TBARS), tocopherol, lipid hydroperoxides, and Vit-C (57).

6,7-DHC not only exhibits the ability to decrease sugar levels in the blood and mitigates the oxidative stress induced by diabetes. However, it demonstrates efficacy in reducing the incidence of diabetic renal disease and related problems. These findings support the potential of 6,7-DHC as a promising sugar-lowering agent (58,59). In a study conducted by Surse et al., it was observed that the treatment of 6,7-DHC at doses of between 0.05 and 0.1 g/kg led to a decrease in the levels of TBARS, circulating sugar, nitrogenous product in blood, and serum creatine metabolite (60).

Additionally, there was an increase in plasma protein levels in "Sprague Dawley" rats with STZ-triggered type 1 diabetes with renal complications. Furthermore, it was observed that therapy with 6,7-DHC inhibits the expression of fibronectin mediated by transforming growth factor-beta 1 (TGF-β1) by mitigating the decrease in PPARγ levels in rats with diabetic nephropathy. The findings of this epigenetic study demonstrated a reduction in bone morphogenetic protein 6 (Bmp6) expression and an increase in matrix metalloproteinase 13 (Mmp13) expression, which are part of complex networks and signalling pathways that play a role in the onset and advancement of diabetes and its complications. This leads towards drawing the opinion that 6,7-DHC may have the capacity to ameliorate the diabetes and renal disease that are linked with it (60).

### 3.6. Antibacterium activity

The transmission of the human disease caused by the O157:H7 strain of Escherichia coli is hypothesized to transpire through exposure to human or pet feces that have been infected, either through passive or active means. The study conducted by Duncan and colleagues investigated the effects of 6,7-DHC, a coumarin produced from plants, on the survival of E. coli O157 in an environment that mimics the circumstances of the gastrointestinal tract. A noteworthy decrease in the viability of the imported E. coli O157 strain was seen when 6,7-DHC was introduced to in vitro digestive system simulations, including the human colon and foregut.

Moreover, in a controlled experimental environment, four young cattle were deliberately infected with E. coli O157 and subsequently administered 6,7-DHC (61). The analysis of stool samples collected after colonization revealed a reduction in the prevalence of the pathogen to 18% among the treated calves, in contrast to the 37% observed in the control group of calves that did not receive 6,7-DHC. This indicates that coumarin compounds found in plants consumed as part of the diet or taken as dietary supplements may possess the ability to inhibit the growth of E. coli O157 in the gastrointestinal tract (62). In another study, the pathogenicity of E. coli O157:H7 was reduced in vivo in the helminth Caenorhabditis elegans, and its Shiga-like toxin type 2 (Stx2) Gene was suppressed by 6,7-DHC. These results suggest that coumarins could be used as part of a virulence factor suppression regimen to combat chronic infections caused by E. coli O157:H7 (63).

### 3.7. Anticancer activity

The application of natural coumarins in the field of cancer chemotherapy has attracted considerable interest owing to their potential as therapeutic agents targeting diverse cancer cell lines, such as human malignant melanoma (HMM) cells G361, oral-origin squamous cancer cell lines, and leukemic monocyte cell lines (64). In their experimental study, Jawun et al. present evidence about the possible anti-cancer effects of 6,7-DHC and elucidate its modes of action against dog mammary adenocarcinomas (commonly abbreviated by CMT) cell lines, namely CF41.mg, and CMT-U27. The study reveals significant inhibitory effects of 6,7-DHC on the livability and migratory capacities of each cell type (5).

Furthermore, the utilization of 6,7-DHC induces an increase in the protein production of cysteine-aspartic acid protease 3, a well-established biomarker of programmed cell death, ultimately leading to the death of apoptotic cells. Also, the administration of 6,7-DHC results in a temporary cessation of the cell cycle advancement in both cell types. Interestingly, the chemical significantly reduces the protein expression of cyclin-dependent kinase 4 and cyclin D1, which serve as regulators of the G1/S transition, in both cell lines. It is worth mentioning that the cessation of the cell cycle takes place at specific stages, specifically the G0/G1 phase for CMT-U27 cells and the S phase for CF41.mg cells. The results of this study shed light on the possible effectiveness of 6,7-DHC as an anti-cancer agent and offer a theoretical basis for future in vivo experiments and potential clinical trials (5,65).

In their experiment, Anand et al. observed that the administration of 0.05 g/kg of 6,7-DHC resulted in the inhibition of cancer cell proliferation. This effect was achieved by the suppression of NF-κB and the Bcl-2 pathway, leading to the induction of cancerous tissue mortality (66). At a dose of 0.05 mg/mL, 6,7-DHC exhibits inhibitory effects on pancreatoid and pulmonary carcinoma, as well as preventive effects on cancers of the gastrointestinal tract. These effects are achieved through the activation of mitogen-activated protein kinase signalling pathways and cysteine-aspartic proteases 3 and 9, ultimately resulting in apoptosis (67).

A study aimed to evaluate the inhibitory effect of 6,7-DHC on tumour formation in the A253 cell line derived from the glandular submandibularis. This assessment involved conducting investigations in both laboratory settings and living systems. Furthermore, the investigation of the anticancer effects of 6,7-DHC on A253 cells and in a cross-species graft model of salivary gland tumours was conducted using several scientific methodologies, with a specific emphasis on assessing cell viability and apoptosis (68,69).

The findings of the study indicated that the administration of 6,7-DHC at doses ranging from 50 to 150 μM had a suppressive effect on the growth of the A253 cell line in an in vitro environment. It is worth noting that the observed effect was dependent on both the dosage delivered and the length of the treatment. Moreover, in the salivary gland, the administration of 6,7-DHC at a dosage of 100 mg/kg/day for 18 days resulted in a noteworthy decrease in tumour cell growth. Therefore, the results indicate that 6,7-DHC exhibits potential as a prospective oral anticancer drug for the treatment of salivary gland cancer (68,70).

### 3.8. Adipocyte differentiation hindering activity

Traditionally, it has been widely acknowledged that fat tissue generated from adipocyte precursor serves as a passive storage site for energy and facilitates thermogenesis in the body. Nevertheless, the adipose tissue, despite its function as a loose connective tissue, is not biologically inactive (71). It operates as a dynamic endocrine organ, generating a range of bioactive compounds including adipose-specific secretory factor, estradiol, adipokine hormone, and tumour necrosis factor-alpha (TNFα). However, an excess accumulation of adipose tissue, particularly in those who are overweight or obese, plays a key role in increasing the likelihood of developing metabolic syndrome. This condition further predisposes affected individuals to a range of disorders that are associated with inflammation (72).

6,7-DHC has been reported to exhibit the ability to improve glucose metabolism and induce controlled fat cell demise by stimulating the mitochondrion pathway, hence beginning a process of cell death in 3T3-L1 adipocytes (71). An additional investigation has emphasized that 6,7-DHC demonstrates anti-adipogenic properties through modulating the peroxisome proliferator-activated receptor gamma (PPAR-γ) and CCAAT/enhancer-binding protein alpha (C/EBPα) via the adenosine monophosphate-activated protein kinase (AMPK) signalling pathway (73).

## 4. Synthesis and biological evaluation of 6,7-DHC's derivatives

The synthesis of 6,7-dimethoxychromen-2-one (1) by methylation from 6,7-DHC was reported by Zhang et al. (74). The overall yield achieved in this process was 74.4%, as seen in Equation 1. In their study, Zhao (75) employed a similar methodology to produce the compound (1). The researchers focused on examining and refining the catalysts used in the methylation process of 6,7-DHC. The use of ionic liquids containing imidazolium cations as catalysts has several advantages, including enhanced reaction yields, improved selectivity, and overall reaction efficiency at lower temperatures (76).

Additionally, these catalysts exhibit the potential for reusability. The upregulation of tissue factor (TF) is a primary contributor to elevated concentrations of oxidised lipoproteins and circulating cholesterol (77). In a biological investigation, it was observed that the compound (1) displayed notable effects on total cholesterol and blood lipid levels, resulting in a subsequent decrease in the expression of TF during thrombus development (78). Additionally, this compound demonstrated strong antioxidant properties. According to a separate research investigation, it was found that the compound (1) exhibited significant inhibitory efficacy against the enzyme clostridium histolyticum collagenase (79).

Adfa *et al.* (83) synthesized a series of scopoletin derivatives by dissolving 3,3-diethoxy-propionic acid ethyl ester (**3**) and different *meta*- and *para*-substituted toluene derivatives in liquid $H_3PO_4$. At 100 °C heating for 120 minutes, different coumarin-based derivatives were synthesized, as illustrated in **Scheme 6**. Based on the microbiological examination, the most potent anti-termite capabilities were demonstrated by scopoletin (**2**), which was followed by 6-methoxy-2-chromenone (**4**), 6-coumarinol (**5**), and umbelliferone (**6**) (84).

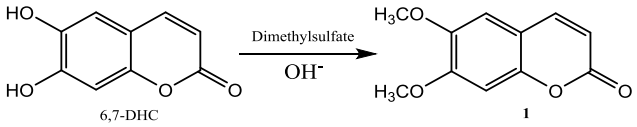
The authors, James *et al.* (85), present an innovative approach for the synthesis of the anandimycin B (**11**) framework, which is commonly found in the gilvocarcin V group of antibiotics. This methodology's primary novelty resides in applying a recently devised benzene ring cyclization method known as the "BHQ Reaction". The synthesis of coumarin (**7**) begins with 6,7-DHC. In this process, an allyl group is attached to carbon 7, followed by the O-methylation of carbon 6 to yield coumarin (**7**) (86). This reaction is facilitated by the catalyst bromallylene, dipotassium carbonate, and propanon. The Claisen-Yaldane rearrangement of (**7**) resulted in the formation of compound (**8**) (87). The trichloroacetyl functionalization of the compound (**8**), followed by the cyclization of the resulting ester crystalline compound (**9**), proceeded without difficulty. This reaction ultimately led to the successful isolation of coumarin (**10**), with a yield of 84% after undergoing column separation analysis, as shown in **Scheme 7** (88).

A recent study was conducted to do a thorough investigation aimed at designing and synthesizing a range of 6,7-DHC derivatives with tailored alterations (89). The alterations encompassed the addition of several nitrogen-bearing moieties at the C7 position of the parent 6,7-DHC structure, along with replacements at positions C4 and C8. This study evaluated the potential hepatitis B suppressant activities of these derivatives. To assess the effectiveness of hepatitis B suppression measures, a series of *in vitro* experiments were conducted utilizing the human hepatoblastoma cell line (HepG2.2.15) (90).

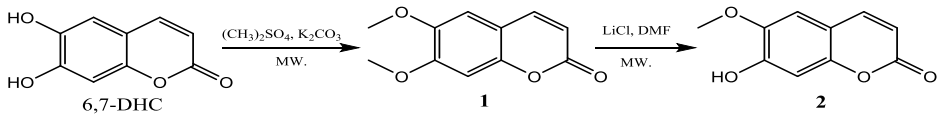
The examination of hepatitis B suppression activity was performed using an enzyme-immunoassay kit, while the evaluation of cytotoxicity was undertaken using the tetrazolium-based viability assay. Lamivudine was utilized as a standard control during these tests. Significantly, the results of these experiments revealed a series of chemicals that showed a notable potential in inhibiting the spread of the hepatitis virus type B (HBV) (91). Significantly, the incorporation of 1-Oxa-4-azacyclohexane moieties led to a noteworthy suppression of hepatitis B e antigenic protein (HBeAg) expression. In a similar vein, the incorporation of the 2-methylglyoxaline moiety led to a notable reduction in the expression of hepatitis B surface antigen (HBsAg) (92).

Out of the compounds (**Scheme 8**) that were subjected to testing, compound (**12**) showed notable potential by exhibiting the highest level of anti-HBeAg activity, with an $IC_{50}$ value of 15.8 µM. Compound (**13**) demonstrated remarkable efficacy in inhibiting HBsAg, as seen by its notably strong anti-hepatitis activity ($IC_{50}$ = 21.4 µM). It is worth mentioning that compounds (**14** and **15**) exhibited a moderate, yet significant, level of efficacy against HBV and displayed suppression of hepatitis antigen (93). In addition, it was shown that compounds (**16**) displayed a modest level of activity against HBV and demonstrated an inhibitory effect on HBeAg. Moreover, these compounds demonstrated improved resistance to metabolic breakdown, hence suggesting their viability as prospective candidates for subsequent advancement. The work provides useful information that contributes to the search for new therapeutic drugs targeting HBV (94).

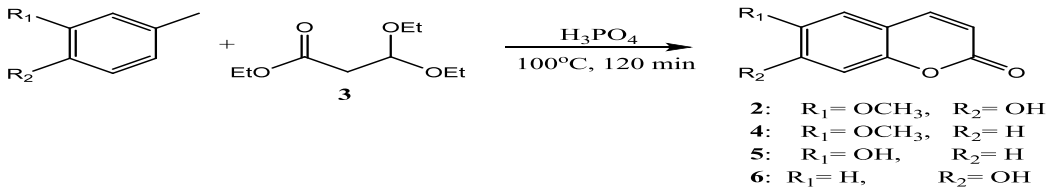
The Mitsunobu-type monoalkylation reaction of natural 6,7-DHC under extreme ultrasound-assisted conditions was established by Cravotto *et al* (95). Several substances with strong psychological effects, or their precursors, were produced from 6,7-DHC, and daphnetin (**17**). Under sonication-driven circumstances, the phytoestrogenic compound (**18**), which was obtained from *Ferula jaeschkeana Vatke* (96), was produced with a good yield. Moreover, straightforward methylation of compound (**19**) produced collinine, a chemical that inhibits viruses and platelets assembly (from *fagara mantchurica whole*). A new SQ-hopanoid cyclase inhibitor could potentially be created via a different selective epoxidization of 7-farnesyle-6-hydroxycoumarin (**20**) (97), as shown in **Scheme 9**.



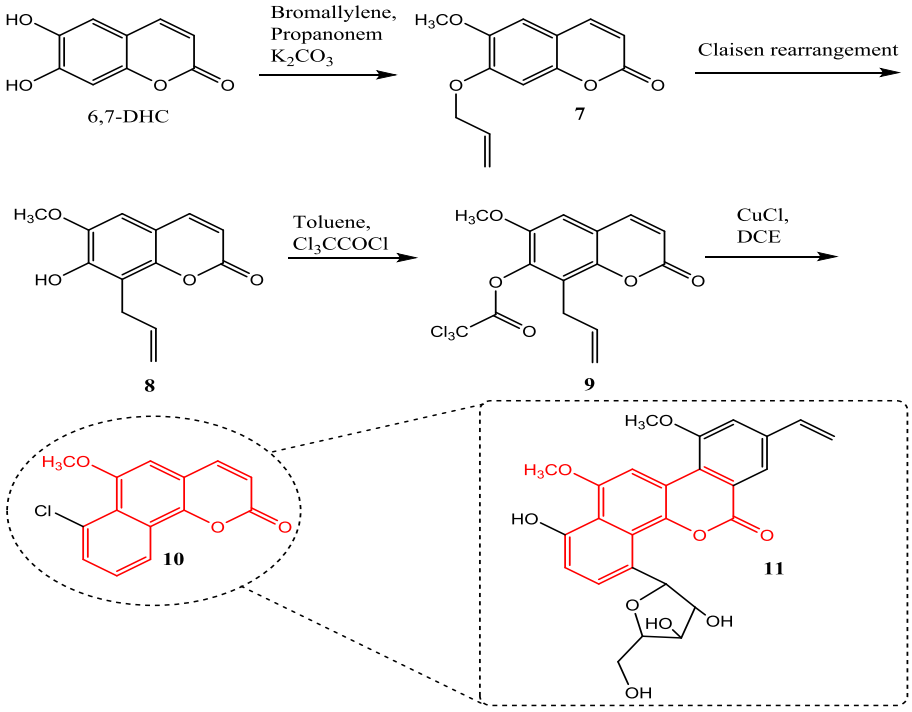

**Equation 1**. Synthesis of 6,7-dimethoxychromen-2-one



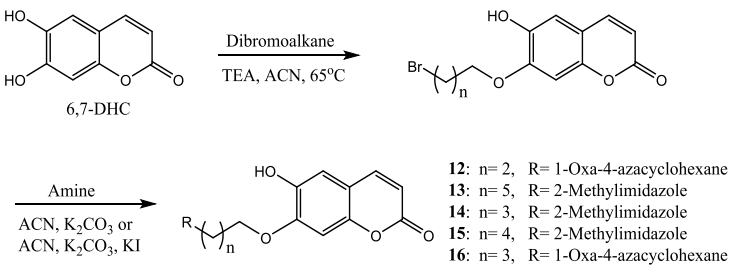

**Scheme 5**. Synthesis route of scopoletin



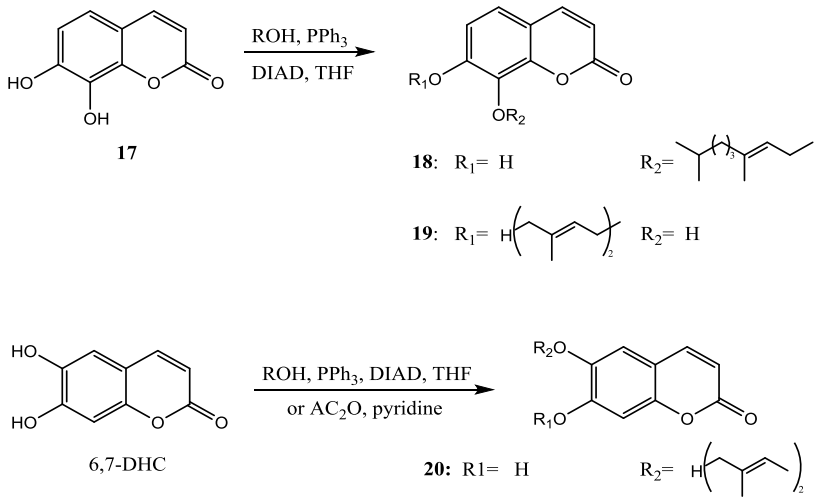

**Scheme 6.** Synthesis route of different coumarin analogue




**Scheme 7**. Synthesis of the anandimycin B skeleton




**Scheme 8.** Synthetic routes of 6,7-DHC derivatives. ACN: acetonitrile, TEA: triethylamine.



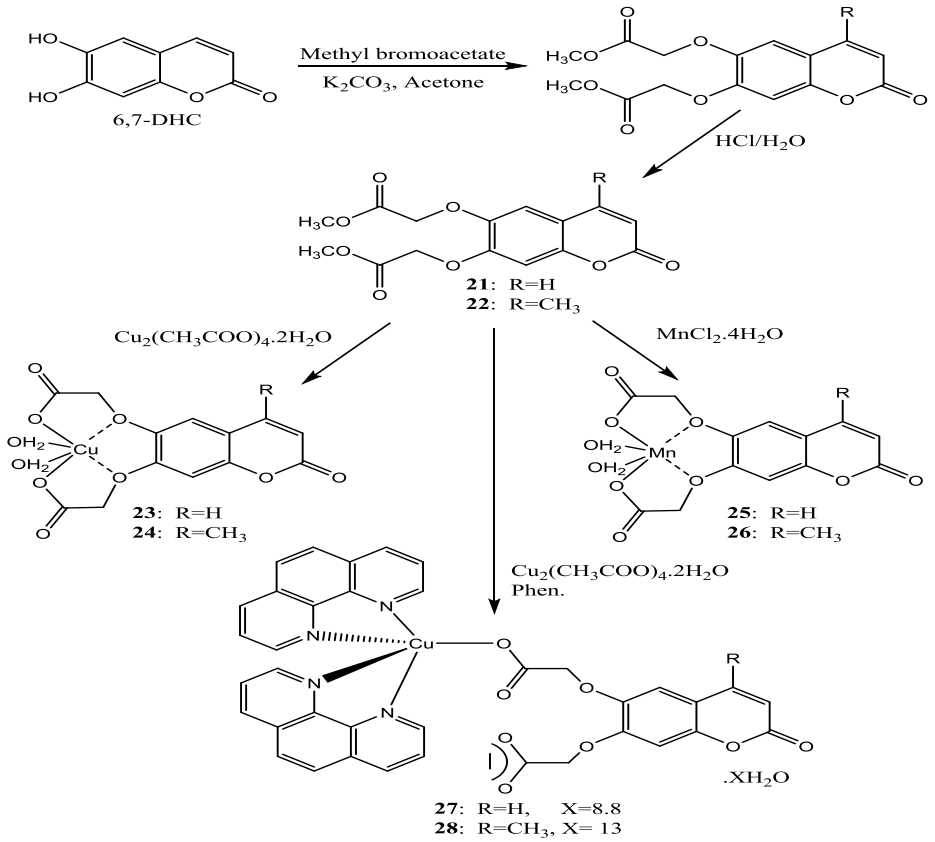

**Scheme 9**. Mitsunobu-type monoalkylation reaction of natural 6,7-DHC. DIAD: Diisopropyl azodicarboxylate, THF: tetrahydrofuran

In their study, Reaven *et al.* (98) utilized two newly developed coordination molecules, namely 2,2'-((2-oxo-2H-chromene-6,7-diyl)bis(oxy))diacetic acid (**21**) and 2,2'-((4-methyl-2-oxo-2H-chromene-6,7-diyl)bis(oxy))diacetic acid (**22**), to proceed reactions with divalent manganese and copper salts, resulting in the formation of the respective metal-ligand systems, as illustrated in **Scheme 10**. The metal complexes exhibited notable antibacterial action against many microbial species, such as drug-resistant *S. aureus*, *Erwinia herbicola*, *colibacilloses*, and *albicans* yeast (99).

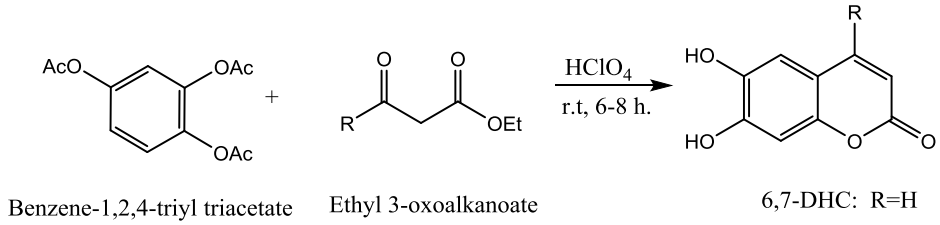
Ligands (**21**) and (**22**), which do not include any metal, show efficacy against all the tested microbial species. Complexes (**23**–**26**) did not exhibit any observable activity when compared to the ligands (**21** and **22**) without metal. However, the phenolic hydroxy adducts (**27** and **28**) showed inhibition against drug-resistant *S. aureus* ($MIC_{80}$ = 12.1 µM), *colibacilloses* ($MIC_{80}$ = 14.9 µM), and *Erwinia herbicola* ($MIC_{80}$ = 12.6 µM). Adduct (**27**) exhibited significant anti-*albican* action with a ($MIC_{80}$) of 22 µM, compared to the readily purchasable antifungal drug ketoconazole with a $MIC_{80}$ of 25 µM (100).

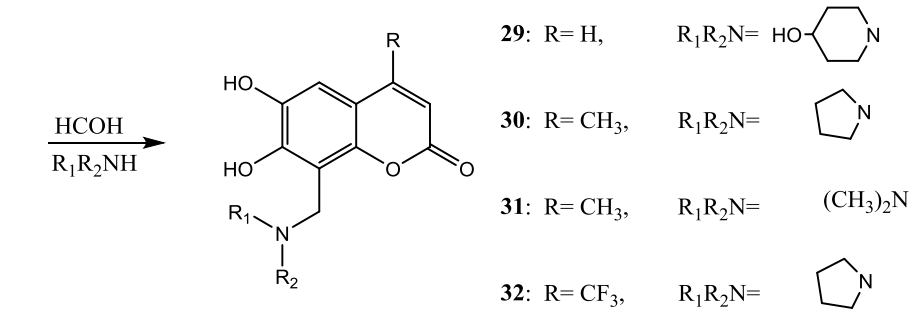
To enhance the physiological stability and antitumor efficacy of 6,7-DHC, a collection of C4 and C8-substituted 6,7-DHCs has been developed and synthesized using benzene-1,2,4-triyl triacetate, ethyl 3-oxoalkanoate, and catalyzed by perchloric acid ($HClO_4$) to accomplish coumarin synthesis, followed by series of Mannich amination reactions to produce compounds (**29**-**32**), as seen in **Scheme 11**, (101). The metabolic half-life of all the recently synthesized compounds was assessed in a human hepatic microsomal S9 Mix, and their *in vivo* anti-tumor activity was examined in A549, and B16 lineages of cells. The assay results indicate that most of the compounds that were created exhibited superior anti-proliferative activity compared to the original chemical, as evidenced by their $IC_{50}$ values being below the micromolar level (102).

Regarding the structural characteristics, the incorporation of an aminoalkyl group at the C8 position is noteworthy. This group possesses an amine moiety that can potentially engage in self-association by H-bonding with the 7-OH group of 6,7-DHC. This interaction may have a substantial impact on both the physiological stability and the anti-cancer activity of the compound (103). Additionally, the introduction of a hydrophobic moiety at the C4 position resulted in a modest enhancement of the antiproliferative activity. On the other hand, a lipophobic group at the C4 position was found to be more advantageous for improving the compound's physiological stability (104,105). Among the examined compounds, the compound (**32**) had the highest potency as a candidate chemical. It demonstrated a nearly 20-fold enhancement in antiproliferative activity ($IC_{50}$ = 1.9 and 3.8 µM for A549 and B16, respectively) and a 3-fold extension in half-life in human liver S9 ($t_{1/2}$ = 115.3 min) when compared to 6,7-DHC ($IC_{50}$ = 48.9 and 17.4 µM for A549 and B16, respectively, $t_{1/2}$ = 44.7 min) (106).




**Scheme 10**. Synthesis of coumarin-based $Cu^{++}$, and $Mn^{++}$ salts




**Scheme 11**. Synthesis of compounds **29** – **32**

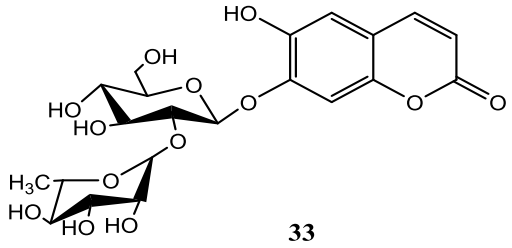
Another group of researchers investigated the free radicals quenching activity of compounds (**29**-**32**) (107). In their study, three different assays—the 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^+$), and ferric reducing antioxidant power (FRAP)-were used to examine the antioxidative properties *in vitro*. These assays are commonly used to determine whether a substance can effectively quench the effects of free radicals (108–110). The outcomes of the total antioxidant capacity (TAC) were reported as the trolox equivalence value, which is written as the number of mmol

equivalents of trolox for every mmol of the sample (mmol trolox/mmol sample) (111). If the TAC value is greater than two, the antioxidant activity is high; if it is between one and two, the antioxidant activity is moderate; and if it is less than one, the antioxidant activity is low. According to the TAC results of compounds (**29**-**32**), these compounds showed high to moderate radical scavenging activities, as shown in **Table 1** (112).

**Table 1**. Free radicals quenching activity of compounds (**33**-**36**)

| Compound | DPPH | $ABTS^{+}$ | FRAP |
|---|---|---|---|
| **29** | 1.580±0.085 | 1.652±0.101 | 1.393±0.073 |
| **30** | 1.210±0.042 | 2.211±0.169 | 1.171±0.038 |
| **31** | 1.720±0.071 | 2.229±0.182 | 1.601±0.116 |
| **32** | 2.350±0.138 | 2.530±0.257 | 1.333±0.073 |

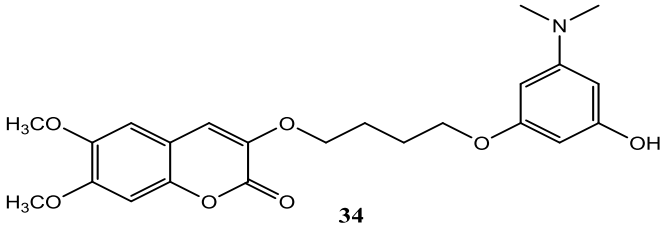
In a research undertaken by Daniil and colleagues (113), a novel 6,7-DHC glycoside (**33**), illustrated in **Figure 2**, was effectively isolated from the blooms of *Calendula prolifera*, a member of the *Asteraceae* family. Subsequent investigations delved into the varied biological features of this compound, revealing its extraordinary competence in suppressing α-glucosidase and amylase enzymes. Furthermore, this molecule revealed a remarkable capacity to reduce the development of advanced glycosylation products, a process inherent to the pyrolysis reaction. These convincing findings collectively underline the promising potential of this compound as a viable candidate for lowering blood sugar, therefore establishing it as a compelling challenger in anti-diabetic therapies (114).




33

**Figure 2**. The structural framework of 6,7-DHC glycoside

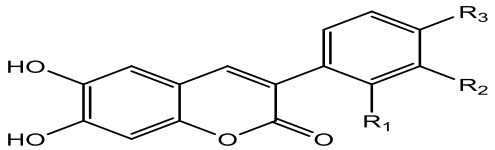
Pisani and colleagues (115) created a broad range of substituted coumarins by synthesizing them and then linking them via an aliphatic chain to the hydroxyl and amine-substituted oxybenzene moiety. These coumarin derivatives were tested to see how well they inhibited the cholinesterase enzymes. It was found that a 6,7-DHC's derivatives that substituted at C3 of coumarin backbone (**34**, as shown in **Figure 3**. had the highest acetylcholinesterase antagonistic potency in the series. The affinity of the derivative (**34**) was exceptional ($IC_{50}$ = 0.236 nm), and it showed extraordinary acetylcholinesterase/butyrylcholinesterase preference (SI > 300,000) (116).




34

**Figure 3**. The structural framework of the derivative **34**

Mahmut *et al.* (117) conducted a thorough investigation into the inhibitory potential of 6,7-DHC's derivatives on serum PON1 aryldialkylphosphatase. This calcium-dependent enzyme hydrolyzes saturated and unsaturated cyclic esters and organophosphorus compounds while protecting lipoproteins with a low density against degradation. Three dihydroxycoumarin derivatives (**35**–**37**), illustrated in **Figure 4**, were thoroughly examined in their inquiry. Interestingly, these compounds showed a significant degree of efficacy with $IC_{50}$ values of 0.012, 0.022, and 0.003 for **35**, **36**, and **37**, respectively, in inhibiting the activity of pure serum PON1. Notable is the determined $IC_{50}$ value of 6,7-DHC, which was measured to be 0.178 mM (118).




| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| **35**: | $CH_3$ | H | H |
| **36**: | H | $CH_3$ | H |
| **37**: | H | H | $CH_3$ |

**Figure 4**. Structural frameworks of compounds **35**-**37**

## 5. Conclusion

Investigations into the chemical construction and pharmacological effects of 6,7-DHC and its related compounds have received considerable attention. Radical quenching activity, which results in reduced amounts of reactive nitrogen and oxygen and species, is one of the many biological effects and molecular principles linked with 6,7-DHC. All sorts of pharmacological actions, including those against cancerous growth, inflammation, and phospholipid-associated syndrome, may be linked together in this way. Chronic inflammation and extreme oxidative damage are major contributors to the development of hepatic cancer from liver cirrhosis. In addition, 6,7-DHC exhibits potential effectiveness in controlling blood glucose levels, combating different invading microorganisms, and hindering adipogenic differentiation. These varied pharmacological effects make it an interesting molecule worthy of further study and possible clinical application. Derivatives can be synthesized using 6,7-DHC's benzopyranone and dihydroxyphenolic structural motifs. These derivatives will broaden the scope of 6,7-DHC's physiological activities. Future studies should focus on expanding therapeutic applicability by learning about pharmacological pathways under laboratory conditions and within a vital system. Increasing therapeutic efficacy, preserving research integrity while creating new 6,7-DHC derivatives, and conducting in-depth mechanistic investigations to prove their clinical viability are all priorities.

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## التوليف والملامح الدوائية لـ 7،6-ثنائي هيدروكسي الكومارين ومشتقاته: مراجعة موجزة


**الخلاصة**

**المقدمة:** يعمل الإسكوليتين، والمشار إليه علميًا باسم 6,7-ثنائي هيدروكسي الكومارين، باعتباره المكون النشط بيولوجيًا الأساسي الموجود في Cortex Fraxini (المعروف باسم لحاء الرماد)، وهي مادة طبية آسيوية قديمة. يستخدم ممارسو الأعشاب الطبقة الخارجية للفرع أو لحاء جذع نبات Cortex Fraxini لخصائصه الطبية اللطيفة والآمنة وإمكاناته كمكون غذائي. في العصر المعاصر، شهد المشهد تحولًا ملحوظًا بسبب ظهور مجموعة واسعة من مشتقات 6,7-ثنائي هيدروكسي الكومارين المبتكرة. أثارت الطفرة الأخيرة في الابتكار اهتمامًا متزايدًا بفهم الآليات الجزيئية التي تكمن وراء تأثيرات Cortex Fraxini و6,7-dihydroxycoumarin في التطبيقات السريرية. **الهدف**: تسعى هذه المراجعة الموجزة إلى تجميع المعرفة الواسعة المتراكمة في العقد الماضي فيما يتعلق بالملامح الدوائية والمبادئ المرتبطة بـ 7،6-ثنائي هيدروكسي الكومارين ونظائره الكيميائية. علاوة على ذلك، فإننا نهدف إلى تقديم نظرة عامة موجزة وشاملة للخصائص الفريدة لـ 7،6-ثنائي هيدروكسي الكومارين. **الاستنتاج**: هذا المشروع إلى تعزيز فهم الإمكانيات المتنوعة التي تقدمها هذه المادة الكيميائية والمركبات المرتبطة بها عبر مجالات البحث والتطبيق المختلفة.


**الكلمات المفتاحية**: إسكوليتين، 7،6-ثنائي هيدروكسي كومارين، كورتيكس فراكسيني، كومارين.