CHAPTER – I
SYNTHETIC APPROACHES AND BIOLOGICAL APPLICATIONS OF COUMARINS: A REVIEW
Synthetic approaches and biological applications of Coumarins: A Review

Coumarins owe their class name to ‘Coumarou’, the vernacular name of the tonka bean (*Dipteryx odorata* Willd., Fabaceae), from which coumarin itself was isolated in 1820.[1] Coumarin is classified as a member of benzopyrone family of compounds.[2] Benzopyrones (Figure 1) were subdivided into benzo-α-pyrone to which the coumarins belong and the benzo-γ-pyrone, of which the flavonoids are principal members.

![Figure 1](image.png)

**Figure 1**

The chemical structures of benzopyrones subclasses

A= Benzo-α-pyrone , B= Benzo-γ-pyrone

Several coumarin derivatives have been found to be widely distributed in the plant kingdom 3-5. They were found at high levels in some essential oils, particularly cinnamon bark oil (7,000 ppm), cassia leaf oil (up to 87,300 ppm) and lavender oil. Plants belong to the natural orders of Orchidaceae, Leguminaceae, Rutaceae, Umbelliferae, and Labiatae are rich sources of naturally occurring coumarins.[6]

Coumarin was initially considered to be a benzoic acid derivative, but the classical approach by W. H. Perkin, Sr.,[7] it was synthesized from salicylaldehyde to form o-hydroxycinnamic acid, which loses a molecule of water in forming the lactone ring. Thus from the point of view of their chemical constitution, a group of lactones derived from o-hydroxycinnamic acids: alternately stated, a coumarin ring system is
formed by the fusion of benzene and α-pyrone ring, i.e., coumarins are classified as oxygenated heterocycles.

### 1.1 Classification of Coumarins:

Coumarins have been generally categorised as follows:

a. Simple coumarins
b. Furanocoumarins
c. Pyranocoumarins
d. Biscoumarins and Triscoumarins
e. Coumarinolignans.

#### 1.1a. Simple coumarins:

Several coumarins have been isolated since the first example was reported in 1820. Murray et al \(^8\) have written an excellent book that provides a comprehensive overview of naturally occurring coumarins (1–9) as shown in Figure 2.

![Figure 2](image-url)
1.1b. Furanocoumarins

Furanocoumarins or Furocoumarins are a class of organic compounds produced by a variety of plants. The chemical structure of furanocoumarins consists of a furan ring fused with coumarin. The furan may be fused in different ways, thus producing several isomers. The compounds that form the core structure of the two most common isomers are Psoralen 10 and Angelicin 11 (Figure 3). Derivatives of these two core structures are referred as linear and angular furanocoumarins. Many furanocoumarins are toxic and are produced by plants as a defense mechanism against various types of predators ranging from insects to mammals. Furanocoumarins have other biological effects as well.9 The structures of different furanocoumarins (10-22) are shown in Figure 3.
1.1c. Pyranocoumarins

Pyranocoumarins are coumarins which contain a pyran ring fused at C-7 or C-8. The Furano and Pyranocoumarins (23–26) (Figure 4), which occur in fruits and roots of Apiaceae and Rutaceae families.

![Figure 4](image)

1.1d. Biscoumarins and Triscoumarins

In recent years several Biscoumarins (27-30) (Figure 5) were isolated from plants. Biscoumarin derivatives were also identified from Rutaceae family and synthesized through expedite methods. Triscoumarins 30 (Figure 5) have been isolated from *Daphne mezereum* and *Daphne oleoides*.

![Figure 5](image)
1.1e. Coumarinolignans

Lignans and neolignans are formed in nature by oxidative dimerization of various C₆–C₃ phenols.¹⁹ Lignans are widely spread in nature and have broad range of biological activities viz, antitumour,²⁰ antifungal etc.²¹ Aquillochin (Cleomiscosin-C) 31 and the regioisomer cleomiscosin-D 32 (Figure 5) are new class of coumarinolignans.²²

![Figure 6](image)

**Figure 6**

1.2. Previous Synthetic approaches for coumarin derivatives:

Coumarins can be obtained from the plants by different extraction methods such as Maceration under sonication, infusion and supercritical fluid extraction ²³. However, the extraction from plants is time consuming & tedious job so there is a need for sophisticated instrument for separation process to get the pure product. Chemically, coumarins can be synthesized by various methods such as the Pechmann reaction, ²⁴a–f Knoevenagel condensation, ²⁵a–d Claisen rearrangement, ²⁶ Perkin, ²⁷a–c Wittig, ²⁸a–d Reformatsky²⁹ and catalytic cyclization reactions ³⁰ etc.

(1) Perkin reaction:

The chemical synthesis of coumarin was first achieved by Perkin⁷. In this reaction Perkin used salicylaldehyde (33) as synthon (Scheme-1). Coumarin was obtained by heating salicylaldehyde with acetic anhydride and anhydrous sodium acetate. This reaction proceeds through the formation of an intermediated o-hydroxy cinnamic acid derivative which converts spontaneously into the lactone ring.
2. Pechmann reaction:

The reaction of a phenol (34) with Malic acid in presence of conc. Sulphuric acid leads to the formation of hydroxyl phenyl coumarin derivatives\textsuperscript{31}. Many substituted phenols do not undergo this reaction; only coumarins unsubstituted in the pyrone ring are obtained (Scheme-2).

3. Pechmann-Duisberg reaction:

Pechmann and Duisberg \textsuperscript{32} found that phenols (36) condense with p-ketonic esters in the presence of sulfuric acid, giving coumarin derivatives (37). This reaction has found extensive applications in the synthesis of various coumarin derivatives (Scheme-3).

4. Knoevenagel reaction: The synthesis of coumarin derivatives obtained from o-hydroxy(aryl/phenyl)aldehydes (38,40) by condensation with ethyl malonate, ethyl
acetoacetate, ethyl cyanoacetate, etc., in the presence of piperidine, pyridine, and other organic bases. 33 (Scheme-4)

5. Hoesch reaction:

Condensation of resorcinol with cyanoacetic ester in acidic medium gives 4, 7-dihydroxycoumarin.34 (Scheme-5)

6. Wittig reaction:

In the Wittig reaction, the alkene formation occurs from carbonyl compounds and phosphonium ylides, proceeding primarily through betaine and/or oxaphosphetane intermediates (Scheme 6). This type of olefination of o-hydroxycarbonyl aromatic compounds, followed by lactonisation, is a well-known method for the preparation of coumarin derivatives.35-42 22 Natural prenylated
coumarins, like coumurrayin and other allylcoumarins were synthesized by expedite synthetic strategies.\textsuperscript{43-45}

\textbf{Scheme-6}

7. Reformatsky reaction

Condensation of aldehydes or ketones with $\alpha$-halo esters (43) in the presence of organozinc derivatives to yield $\beta$-hydroxy esters is known as the Reformatsky reaction (Scheme 7).

\textbf{Scheme-7}

In appropriate reaction conditions, lactonisation could occur with the formation of coumarins. As a case in point, one must refer the synthesis of 4-cyclohexylhydroxy coumarins via the Reformatsky reaction.\textsuperscript{46}

8. Anschütz Approach \textsuperscript{47,48}:  

\textsuperscript{9}
Condensation of sodium derivative of acetoacetic ester with o-acetoxybenzoyl chloride (45) in ethereal solution yields 4-hydroxycoumarin (Scheme-8).

\[
\begin{align*}
\text{O} & \quad \text{CO} \quad \text{C} \\
\text{O} & \quad \text{C} \quad \text{O} \\
\text{C} & \quad \text{2} \quad \text{H} \\
\text{5} & \quad \text{O} \\
\end{align*}
\]

\[
\begin{align*}
\text{Cl} & \quad \text{O} \\
\text{Na} & \quad \text{O} \\
\text{C} & \quad \text{2} \quad \text{H} \\
\text{5} & \quad \text{O} \\
\end{align*}
\]

\[
\text{O}
\]

\[
\begin{align*}
\text{O} & \quad \text{C} \quad \text{O} \\
\text{C} & \quad \text{2} \quad \text{H} \\
\text{5} & \quad \text{OH} \\
\end{align*}
\]

\[
\begin{align*}
\text{45} & \quad \text{+} \quad \text{46} \\
\end{align*}
\]

Scheme-8

9. Weiss and Merksammer Reaction \(^{49}\):

Resacetophenone (47) on condensation with ethyl ethoxymethylene acetoacetate in presence of alcoholic sodium ethoxide gave 7-hydroxy-3,6-diacetylcoumarin. This method was further extended by Weiss and Kratz\(^{50}\) by taking ethyl ethoxymethylene malonate, similarly condensed to give coumarin-3-carboxylates from resorcinol derivatives (Scheme -9).

\[
\begin{align*}
\text{HO} & \quad \text{O} \\
\text{OH} & \quad \text{COOC} \quad \text{C} \quad \text{2} \quad \text{H} \\
\text{5} & \quad \text{R} \\
\end{align*}
\]

\[
\begin{align*}
\text{RO} & \quad \text{C} \quad \text{2} \quad \text{H} \\
\text{5} & \quad \text{O} \\
\end{align*}
\]

\[
\begin{align*}
\text{OH} & \quad \text{O} \\
\text{HO} & \quad \text{C} \quad \text{R} \\
\text{CO} & \quad \text{C} \quad \text{2} \quad \text{H} \\
\text{5} & \quad \text{K} \\
\end{align*}
\]

\[
\begin{align*}
\text{47} & \quad \text{+} \quad \text{48} \\
\end{align*}
\]

Scheme-9

10. Baker et al. \(^{51}\) observed that α-formylphenyl acetonitrile and its derivatives condense with resorcinol (34) or other phenols, in presence of phosphorus oxychloride or dry hydrogen chloride as condensing agents, leading to produce 3-phenylcoumarins (Scheme-10).

\[
\begin{align*}
\text{HO} & \quad \text{O} \\
\text{OH} & \quad \text{C} \quad \text{2} \quad \text{H} \\
\text{5} & \quad \text{R} \\
\end{align*}
\]

\[
\begin{align*}
\text{RO} & \quad \text{C} \quad \text{2} \quad \text{H} \\
\text{5} & \quad \text{CN} \\
\text{R} & \quad \text{3} \\
\end{align*}
\]

\[
\begin{align*}
\text{HO} & \quad \text{O} \\
\text{OH} & \quad \text{C} \quad \text{6} \quad \text{H} \\
\text{5} & \quad \text{R} \\
\end{align*}
\]

\[
\begin{align*}
\text{49} & \quad \text{49} \\
\end{align*}
\]

Scheme-10
11. Smith and Dobrovolny\textsuperscript{52} observed that 3-carbethoxy 5, 7, 8-trimethyl-6-hydroxycoumarin is produced when duroquinone (50) reacted with ethyl sodiomalonate in benzene solution (Scheme-11).

![Scheme-11](image)

12. Chakravarti and Majumdar\textsuperscript{53} have developed a method by which 3,4-dialkyl-substituted coumarins (53) (not available by the usual methods) may be synthesized from o-hydroxyaryl alkyl ketones, under the conditions of the Reformatsky reaction, are ultimately converted into coumarin derivatives (Scheme-12).

![Scheme-12](image)

13. Bert et al.\textsuperscript{54} approach: Condensing phenolic ethers with CH$_2$ClCH=CHCl either by the Friedel-Craft's reaction or in the presence of zinc dust to obtain ROC$_6$H$_4$CH$_2$CH=CHCl, this is then converted into the corresponding coumarin in two ways, as shown in Scheme-13.
14. Kostanecki acylation of o-hydroxyketones: Kostanecki and Rozycki \(^{55}\) showed that the products obtained by Nagai \(^{56}\) and Tahara \(^{57}\) by heating resacetophenone and its monomethyl ether with acetic anhydride and sodium acetate were chromone derivatives by losing water molecule in the intermediate acyl derivative (Scheme-14).

15. M. S. Manhas et al. \(^{58}\) developed an energy-efficient protocol for solvent-free reactions that are mildly exothermic but not spontaneous. The exothermic reaction mixture is exposed for about 30 sec to low power (about 200 W) microwaves (Scheme-15). After this short burst of energy, the exothermic reaction gets initiated and proceeds on its own to completion. A number of coumarins were synthesized by the Pechmann reaction using this strategy.
16. M.K. Potdar et al.\textsuperscript{59} reported neutral ionic liquids with catalytic amount of acid have been employed for coumarin synthesis via Pechmann condensation of phenols and ethyl acetoacetate under ambient conditions (Scheme-16). The reaction was also successfully carried out at high temperature in 1-butyl-3-methylimidazolium hexafluorophosphate ionic liquid, without any acid catalyst.

\begin{equation}
\text{Scheme-16}
\end{equation}

17. 1-Butyl-3-methylimidazolium chloroaluminate, [bmim]Cl·AlCl\textsubscript{3}, \(N=0.67\) and 1-butylpyridinium chloroaluminate, [bpy]Cl·AlCl\textsubscript{3}, \(N=0.67\) ionic liquids were found to work well as the Lewis acid catalyst and solvent in the Knoevenagel condensations of benzaldehyde and substituted benzaldehydes. J. R. Harjani \textit{et al.}\textsuperscript{60} reported that, in the case of 2-hydroxyarylaldehydes, the reactions led to the formation of 3-ethoxycarbonyl coumarins under ambient conditions (Scheme-17).

\begin{equation}
\text{Scheme-17}
\end{equation}

18. B. Tyagi \textit{et al.}\textsuperscript{61} described the nano-crystalline sulfated-zirconia catalysts, prepared by one-step as well as two-step sol–gel technique, showed excellent catalytic activity with a high substrate to catalyst weight ratio for the synthesis of 7-substituted 4-
methyl coumarins via solvent free Pechmann reaction (Scheme-18). The \( m \)-amino phenol was found to be more reactive than \( m \)-hydroxy phenol.

\[
\begin{array}{c}
\text{R-OH} + \text{COOC_2H_5} \xrightarrow{\text{SZ, } \Delta} \text{R-OCH_3 + C_2H_5OH} \\
\end{array}
\]

\textit{Scheme-18}

1.3. Metabolism of coumarins

Traditionally coumarin has been viewed as the ideal model for studying the complex metabolism of a structurally simple organic molecule, and as such, its metabolic fate has been widely researched.\textsuperscript{62-64} Determining the metabolic nature of coumarin is important in order to utilise the fact that it is metabolised at several sites, and to access the possible dependence of coumarin-induced toxicity on metabolism.\textsuperscript{65,66}

There are several pathways for involved in coumarin in human metabolism (Figure 7). Initially, coumarin is metabolised by specific cytochrome P-450-linked mono-oxygenase enzyme (CYP2A6) system in liver microsomes, resulting in hydroxylation to form 7- hydroxycoumarin. After 7-hydroxylation, coumarin undergoes a phase II conjugation reaction resulting in a glucuronide conjugation associated with 7-hydroxycoumarin. The 7-hydroxylase activity is exceptionally high in human liver microsomes compared with its activity in the livers of other animal species. The activity of coumarin 3-hydroxylase is very high in rodent microsomes but is absent in human microsomes. Coumarin may be metabolised by hydroxylation at all six possible positions (i.e. carbon atoms 3, 4, 5, 6, 7 and 8) to yield (35,58-62) 3-, 4-, 5-, 6-, 7- and 8-hydroxycoumarins (3-, 4-, 5-, 6-, 7- and 8-HCs) and by opening of the lactone.
ring to yield various individual products including \( \text{o-hydroxyphenylacetaldehyde} \) (\( \text{o-HPA} \)) (69, a major metabolite of coumarin in rat and mouse liver microsomes), \( \text{o-hydroxyphenylethanol} \) (\( \text{o-HPE} \)), \( \text{o-hydroxyphenylacetic acid} \) (\( \text{o-HPAA} \)) and \( \text{o-hydroxyphenyllactic acid} \) (\( \text{o-HPLA} \)). Additional metabolites of coumarin include \( \text{6,7-dihydroxycoumarin} \) (\( \text{6,7-diHC} \)), \( \text{o-hydroxyphenylpropionic acid} \) (\( \text{o-HPPA} \)), \( \text{o-coumaric acid} \) (\( \text{o-CA} \)), and \( \text{dihydrocoumarin} \) (\( \text{DHC} \)).

The most common routes of hydroxylation are at positions 7 and 3 to yield \( \text{7-hydroxycoumarin} \) and \( \text{3-hydroxycoumarin} \), respectively (Figure 7). 7-hydroxylation has received the most attention among the various metabolic steps, predominantly because it is the major metabolic route in humans and is easily analysed. Hydroxylation at carbon 3 results in further metabolism via ring opening and cleavage of the carbon 2 atom to yield carbon dioxide. The first step in coumarin metabolism by the latter pathway is the formation of a coumarin 3, 4-epoxide intermediate (67). However, under aqueous conditions, coumarin 3, 4-epoxide degrades rapidly, with the loss of carbon dioxide to form \( \text{o-HPA} \), which can be further metabolized to \( \text{o-HPE} \) and \( \text{o-HPAA} \). But \( \text{o-HPA} \) formation did not accurately reflect the rate of coumarin 3, 4-epoxidation. The expression of CYP enzymes (e.g. CYP2A6) varies between individuals due to genetic and environmental factors. These factors produce inter-individual variation in the metabolism of drugs such as coumarin. The frequency of poor metabolisers varies between species, races and ethnic groups. It has been shown that there exists large inter-species and inter-individual variability in the activity of these enzymes.68
The metabolism of coumarin has been investigated in vivo and in vitro in a wide range of species including humans. Human metabolic studies usually involve oral dosage followed by urine collection with or without timed fractionation. Analysis is carried out by a number of techniques including spectrofluorimetry, HPLC and capillary electrophoresis. Recent in vitro systems employed include tissue slices, hepatocytes, subcellular fractions, and purified and cDNA-expressed enzymes. In the majority of human subjects studied coumarin is extensively metabolised to 7-
The measurement of 7-hydroxycoumarin following an oral dose of coumarin has been employed as a biomarker of human hepatic CYP2A6, the cytochrome P-450 (CYP) isoform which is responsible for coumarin 7 hydroxylation in human liver. Some individuals can metabolise a considerable proportion of coumarin through pathways other than 7-hydroxylation such as the 3,4-epoxidation pathway to α-HPAA.

In humans, there are three genes in the CYP2A subfamily, however, CYP2A6 is mainly of greater importance, as the other two gene products (CYP2A7 and CYP2A13) are either inactive or are not expressed in the liver. CYP2A6 codes the enzyme catalysing coumarin 7-hydroxylation (about 10% of total P450). Recently, CYP2A6 has been reported to be polymorphically expressed in the human liver. It has been shown that CYP2A6 participates in metabolism of nicotine and its metabolite cotinine. Some drugs and chemicals, including coumarin, which is widely used as a probe substance for CYP2A6 both in vitro and in vivo, are also metabolised by this enzyme. Substrates and inhibitors currently known to be metabolised by or interfere with CYP2A6 in vitro and in vivo have been summarised by Pelkonen. Although 7-hydroxycoumarin is the main human metabolite, other hydroxylation pathways are important in humans and, as such, the therapeutic relevance of non-7-hydroxymetabolites should be examined.

1.4. Coumarins as promising lead molecules/drugs

Natural as well as synthetic coumarins have recently drawn much attention due to their numerous therapeutic applications including phototherapy, antitumor, anti-HIV, antibacterials, anti-inflammatory, anti-coagulants. 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. However, the details of relationship between the structure and activity of coumarins remain obscure. It is therefore useful to build up some correlations with the data available in order to better explore the structure activity relation of coumarins in the medicinal chemistry.

1.4.1. Antimicrobial activity

The random use of antibiotics has led to many bacterial strains becoming drug resistant. Development of new and effective antibiotic compounds to target resistant microorganisms has become seriously important and the new products in development should improve these concerns.

The antibacterial properties of coumarins were first recognised in 1945 when Goth et al. conducted an investigation with dicoumarol and it was found to inhibit the growth of several strains of bacteria. All the methods reported in the study of the antibacterial activity of coumarins were disc diffusion methods. Dadák and Hodak suggested that coumarins possess antibacterial activity act selectively against Gram-positive microorganisms. Melliou et al. studied the antibacterial activity of pyranocoumarins using an agar disc diffusion method.

Although most of the natural coumarins have been isolated from the higher plants, some of them have been discovered in microorganisms. Novobiocin (72), clorobiocin (73), and coumermycin A1 (74) (Figure 8) are all members of the coumarin family of antibiotics, have isolated from diverse Streptomyces species and exhibit a potent activity against Gram-positive bacteria.
Chlorobiocin differs from novobiocin in that the methyl group at the C-8 of the coumarin ring is replaced by a chlorine atom, and the carbamoyl at the 3′ of the noviose is substituted by a 5-methyl-2-pyrrolcarboxyl group. Coumermycin A1 contains two of the coumarin-noviose core joined by a 3-methyl-2,4-dicarboxyl pyrole linker and has the same substituted noviose as in chlorobiocin. These compounds target the bacterial enzyme DNA gyrase and inhibit the enzyme-catalyzed hydrolysis of ATP. Each compound contains an individual noviosyl sugar component that imparts the functionality which was essential for biological activity. Removal of the carbamoyl group from novobiocin or its transference to 2-hydroxy group of novobiose, leads to a complete loss of activity. However, replacement of the carbamoyl group with a 5-methyl-2-pyrrolylcarbamoyl group (which is present in coumermycin), leads to >10-fold increases in both anti-bacterial activity and in vitro activity against DNA gyrase. The aminocoumarin antibiotics viz) novobiocin and clorobiocin consist of a 3-
amino-4, 7- dihydroxycoumarin (ADHC) moiety flanked on one side by L-noviose and on the other side by a 3-dimethylallyl-4-hydroxybenzoyl (DMAHB) moiety. Both ADHC and L-noviose are essential for anti-bacterial activity and that the substituents attached to these fragments have a significant impact on their bioactivities.81

Angellicin (11) (Figure 9), naturally occurring first angular furanocoumarin,82 which showed anti-fungal activity, was considered to be a lead structure for a group of synthetic coumarins. Simple long chained hydrocarbons are connected to the furanocoumarin skeleton of angelicin, and the anti-microbial activities are more effective than other furanocoumarins, protection of 6-OH by groups that change the electronic contribution of oxygen 6 to the ring, or change the polarity of the functional groups to a favoured pattern improves the anti-fungal activity.83 Studies have shown that a free 6-OH moiety in the coumarin nucleus was a necessity to combat bacteria.

Systemic analysis of the structural activity relationships have shown that coumarins with a methoxy function at C-7 and an OH group at either the C-6 or C-8 position were invariably effective against a broad spectrum of bacteria. The presence of an aromatic dimethoxy arrangement was shown to be favourable against those microorganisms which required special growth factors (beta-hemolytic streptococcus, streptococcus pneumoniae and haemophilus influenza.84
The coumarins Osthol (26), Scopoletin (75) and Phebalosin (76) (Figure 9), isolated from Rutales species were assayed for the inhibition of the glycolytic enzyme T. cruzi glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Due to some limitations of these novobiocin compounds, particularly with regard to solubility, toxicity and development of resistance, some synthetic coumarins were screened for their antimicrobial and antifungal activity.

Laurin et al. synthesized novel coumarin derivatives 77-79 (Figure 10) and screened against E. coli DNA gyrase supercoiling. Most of the novel analogues exhibited more potent inhibitory activity than novobiocin. The oxime ether 65 exhibited the best antibacterial activity against novobiocin-resistant strains.

Khan and co-workers have introduced oxygenated tricyclic coumarins (80) (Figure 11). These newly synthesized compounds have been showing activity against two bacterial and two fungal strains viz. Micrococcus aureus, Pseudomonas asperigillus fumigatus and Penicillium wortmanni.
Recently, Chimenti et al have prepared N-substituted-2-oxo-2H-1-benzopyran-3-carboxamides 81 (Figure 11) and their derivatives exhibited antibacterial activity against H. pylori metronidazole resistant strains in the range of 0.25–1 μg/mL MIC.\textsuperscript{97}

1.4.2. Anticancer activity

Chemotherapy is the treatment of cancer with anticancer drugs, and its main purpose is to eradicate cancer cells. Applications of coumarin in cancer chemotherapy, was initiated by the application of Warfarin sodium 82 (Figure 12) on V2 cancer cells, granulocytes, lymphocytes and macrophages in animal models.\textsuperscript{98} Among the coumarins screened for anticancer activity, Geiparvarin 83 (Figure 12) was found to be the most representative natural coumarin, isolated from the leaves of Geijera parviflora Lindl which is known for its significant \textit{in vitro} cytotoxic activity.\textsuperscript{99} The compound is constituted of three units: a furan-3(2H), an unsaturated alkenyloxy substituent and a coumarin moiety. The first mentioned unit is necessary for the activity as it could work as an alkylating agent of bionucleophiles, through a Michael-type reaction. In that way, geiparvarin became a challenging lead compound, for the synthesis of analogues that could also be promising candidates for anticancer activity. Thus, geiparvarin analogues were synthesised, in order to determine the structural features (SAR) that account for its \textit{in vitro} cytotoxic effects.\textsuperscript{100-102} Among
these, compound 84 (Figure 12) was the most potent, exhibiting a good selective and inhibitory activity.

![Figure 12](image) Neo-tanshinlactone (85) (Figure 13) is a steroid-like tetracyclic natural product originally isolated from Chinese traditional medicine Tanshen, showed significant inhibition against two estrogen receptor positive human breast cancer cell lines and was 10-fold more potent and 20-fold more selective as compared to tamoxifen. Compound 86 (Figure 13), a congener of 85, is about twice as active as 85 against SK-BR-3 cell line. It may be more structurally complex structure of 85 than is necessary for optimal pharmacologic effects. A complex lead compound may have a simpler pharmacophoric moiety underlying within its structure, and if this pharmacophore can be clearly defined and “dissected out”, the resulting biologically active, simpler molecule may have improved synthetic tractability and be more useful as a scaffold for further analogue design. Lee and co-workers studied the individual contribution (as shown in Figure 13 as structure 87) of the A-, C-, and D-rings of 85 to the selective activity against breast cancer cells and demonstrated that aromatic rings A and D were important for the activity. Importantly, they revealed that ring C could be opened through hydrolysis of the ester bond while keeping the desired biological activity.
Sashidhara et al have also synthesized a small library of novel benzocoumarin derivatives (88-89) (Figure 13) based on naturally occurring neotanshinlactone scaffold and evaluated for their antiproliferative activities against breast cancer cells MCF-7 & MDA-MB-231. A number of derivatives showed good anti-breast cancer activity, in some cases higher to that of the reference compound tamoxifen. One more series of coumarin-chalcone hybrids (90) (Figure 13) were synthesized by using hybrid approach paradigm and evaluated them for in vitro cytotoxicity against a panel of four human cancer cell lines, KB (Oral squamous cell carcinoma), C33A (cervical carcinoma), MCF-7 (Breast adenocarcinoma), A549 (lung carcinoma) and one normal fibroblast NIH3T3 (Mouse embryo fibroblast). Some of the synthesized coumarin-chalcone hybrids were found selective to breast cancer and cervical carcinoma.

Novobiocin 72 (Figure 14) is a member of the coumermycin family of antibiotics and is well established inhibitor of DNA gyrase. Novobiocin derivatives 4-
deshydroxynovobiocin (DHN1) 91 and 3-descarbamoyl-4-deshydroxynovobiocin (DHN2) 92 (Figure 14) were evaluated their ability to inhibit the growth of ErbB2 (Erythroblastic leukemia viral oncogene homolog 2) and SKBr3 breast cancer cell lines. Both the compounds DHN1 and DHN2 exhibited improved activity as compared to novobiocin at 700 μM concentration.107

Figure 14

Terpenoid coumarins auraptene (7-geranyloxy coumarin) 93 and Umbelliprenin 94 (Figure 15) were isolated from plants of the Ferula species and proved to inhibit tumor promoter 12-0-tetradecanoylphorhol-13-acetate (TPA)-induced Epstein-Barr virus (EBV) by the mechanism of suppressing O2- generation in leukocytes.108-109 These coumarins significantly inhibited EBV-EA activation and preserved the high viability of Raji cells. SAR studies, indicate the presence of a prenyl moiet in the terpenoid for its anti-tumor activity.110
In addition, Auraptene significantly reduce the growth and number of metastatic lung tumors in mice bearing B16BL6 murine melanoma. Metastatic pigmented malignant melanoma (M4Beu) cell-proliferation is inhibited by umbelliprenin (IC₅₀ = 12.3μM) via a G1 cell-cycle arrest and through the induction of caspase-inpentent apoptosis. These results indicate that the cytotoxic effect of umbelliprenin is markedly more pronounced in M4Beu cells than in primary fibroblasts, suggests it could be used as a potential therapeutic agent.

Moreover, the inhibitory effects of numerous simple coumarins, synthesized or isolated from plants, on cytotoxic activity have also been reported.

### 1.4.3. Tuberculosis activity

Tuberculosis (MTB or TB) is a common and in some cases, deadly infectious disease, caused by various strains of mycobacteria, usually *Mycobacterium tuberculosis* in humans. Tuberculosis usually attacks the lungs but can also affect other parts of the body. It is spread through the air when people who have active MTB infection cough, sneeze, or spit. Most infections in humans result in an asymptomatic, latent infection, and about one in ten latent infections eventually progresses to active disease, which, if left untreated, kills more than 50% of its victims. Pyranocoumarin (95) (Figure 16) compounds were identified to embody a novel and unique pharmacophore for anti-TB activity. Libraries of coumarin derivatives were synthesized and evaluated for their anti-TB activity in primary screening assays.
A set of coumarin-4-acetic acid benzylidene hydrazides (96) (Figure 16) were synthesized and evaluated for their anti-tubercular activity against *Mycobacterium tuberculosis* H$_{37}$Rv strain using the BACTEC 460 system to determine percentage inhibition.\textsuperscript{123}

### 1.4.4. Antioxidant activity

Coumarins have important effects in plant biochemistry and physiology, act as antioxidants, enzyme inhibitors, precursors of toxic substances, powerful chain-breaker, and can prevent free radical injury by scavenging reactive oxygen species. For monohydroxycoumarins, their anti-oxidant properties have been related to radical-scavenging activity, and inhibition of tyrosine kinases.\textsuperscript{124} But, accumulating data of studies reveal that dihydroxycoumarins are not only effective inhibitors of Fe$^{3+}$ ascorbate-dependent microsomal lipid peroxidation and aqueous alkylperoxyl radicals, but also scavengers of superoxide anion radicals, and better antioxidants than monohydroxycoumarins. In addition, the OH groups positioned near C6 and C7 in the coumarin skeleton play an important role in the inhibition of the mushroom tyrosinase.\textsuperscript{125} Coumarin derivatives (coumarin, 4-HC, 7-HC, esculetin, scopoletin, DHC, 4-methylesculetin, and 7-hydroxy-4-methylcoumarin) were analysed for their anti-oxidant properties and their ability to scavenge free radicals. The results showed that esculetin was the most potent radical scavenger, followed by 4-
methylesculetin. The number of hydroxyl groups on the coumarin ring structure correlates with the ROS suppressor function. The structure-based molecular modeling revealed interactions between coumarins and the molybdopterin region of xanthine oxidase (XO). The carbonyl pointed toward the Arg880, and the ester O atom formed hydrogen bonds with Thr1010. Esculetin, which bears two hydroxyl moieties on its benzene rings, had the highest affinity toward the binding site of XO, and this was mainly due to the interaction of 6-hydroxyl with the E802 residue of XO. The chemical structure of scopoletin is similar to that of esculetin except with methoxy moiety substituted for the 6-hydroxyl, which results in a diminished potency of scopoletin to inhibit XO. This further enhances the hypothesis that the H atom of the 6-hydroxyl plays a more significant role than the O atom.

Although the anti-oxidant activity has been primarily attributed to the presence of free hydroxyl groups, a significant anti-oxidant effect has also been reported for compounds where these groups are acetylated. A novel enzyme in the microsomes of the liver catalyzed the transfer of acetyl groups from acetylated polyphenols to certain receptor enzyme proteins which could putatively result in the modification of their catalytic activities. Protein transacetylase (TAase) was found to catalyze the transfer of the acetyl group from DAMC (7,8-diacetoxy-4-methylcoumarin, 97) (Figure 17) to GST. This results in the acetylation of several lysine residues in its active site and subsequent inhibition of the catalytic activity of GST (glutathione S-transferase). Twelve acetoxy coumarins and dihydrocoumarins bearing a phenyl ring and methyl group at C-4 were involved into the experiments. The results of acetylation of GST by the coumarin derivatives by TAase showed that DAMC had the highest catalytic activity, and that acetoxy 4-phenylcoumarins (98) (Figure 17) had significantly less activity. Similar results were obtained when the TAase catalyzed activation of nicotinamide adenine dinucleotide phosphate-oxidase.
(NADPH) cytochrome reductase assay and Aflatoxin B1 (AFB1)-DNA binding inhibitory assay were performed with 4-phenylcoumarins and 4-methylcoumarins.

Figure 17

These results confirmed the hypothesis that DAMC was found to modulate the activities of enzymes including cytochrome P-450-linked mixed-function-oxidase (MFO), NADPH cytochrome c reductase and cytosolic GST when catalyzed by TAase. To elucidate the structure activity relationship (SAR) of the phenyl ring on the benzopyran nucleus, Kumar et al compared the specificities of the acetylated coumarins, biscoumarins, chromones, flavones/isoflavones and xanthones for TAase activity. The results demonstrated that the presence of the phenyl ring on the pyran nucleus of polyphenolic acetates drastically reduces their specificity to TAase modification.

In addition, a variety of plant-derived and various synthetic simple coumarins with different hydroxyl groups and other substituents were tested for their antioxidant activity viz), in relation to the ability to inhibit lipid peroxidation and to scavenge reactive species, for instance, hydroxyl, superoxide radicals and hypochlorous acid. Several coumarins have shown beneficial biochemical profiles in relation to pathophysiological processes dependent upon reactive oxygen species. Classic and three dimensional (3-D) QSAR analysis of radical scavengers, structurally based on coumarin, have also been performed. The photodynamic damage prevention done by some hydroxycoumarins was evaluated, and compared
with that of \textit{p}-aminobenzoic acid (PABA) as a model sun screen. The activity could be related to their antioxidant action which could minimize skin photoaging.\textsuperscript{144}

\textbf{1.4.5. Anti-inflammatory activity}

Inflammation is the process by which leucocytes and materials derived from the serum are directed to the site of tissue injury. In the skin, inflammation is characterized by local redness and swelling. Clotting factors, as well as factors that alter vascular permeability, aid in bringing about vasodilation and enhanced blood flow to the affected area, enhanced permeability of the capillaries and increased migration of effector cells (leucocytes) from the circulatory system to the connective tissue. In the 1970's, it was found that Aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) block the biosynthesis of prostaglandins (PGs), which contribute to a variety of physiological and pathophysiological functions.\textsuperscript{145}

It has been found that several coumarins isolated from plants or of synthetic origin possess significant anti-inflammatory and/or analgesic activities.

Osthole (26) (Figure 18), a natural coumarin, isolated from many medicinal plants, such as \textit{Cnidium monnieri} (Umbelliferae) and \textit{Angelica pubescens}, was tested in cyclooxygenase and 5-lipoxygenase bioassays and turned out to be a moderate and selective a 5-lipoxygenase inhibitor (IC\textsubscript{50} = 36.2 μM).\textsuperscript{146} 4-methylesculetin and 4-methyldaphnetin were tested on ionophore-activated rat leukocytes (a cell system that express both cyclooxygenase and 5-lipoxygenase pathways of arachidonate metabolism) and were found to inhibit selectively the proinflammatory 5-lipoxygenase enzyme with 5, 7- dihydroxy-4-methylcoumarin demonstrating a higher potency against cyclooxygenase.\textsuperscript{147} It is well known that lipoxygenase (LOX) possess regiospecificity during interaction with substrates and on this basis have been primarily designed as arachidonate 5-, 12-, and 15-LOX. 5-LOX represents a dioxygenase that possesses two distinct enzymatic activities leading to the formation
of LTA4, which is converted to 5-hydroxyeicosatetraenoic acid (5-HETE) or five lipooxygenase activating protein (FLAP).

![Figure 18](image)

Esculetin (66) (Figure 18) can selectively inhibit LOXs with different IC$_{50}$. In platelet, esculetin inhibit 12-LOX with IC$_{50}$ = 0.65 μM. Whereas esculetin inhibits the formation of 5-HETE with IC$_{50}$ = 1.46mM in polymorphonuclear leukocytes, although more strongly than HHT (IC$_{50}$ = 57.3mM). The roles of 15-LOX in cancer development are unclear, however, esculetin can also inhibit the formation of 15-HETE from 15-LOX. On investigation of specific products of the LOX pathway mediating the autoregulatory effect of glucose and glucose transport in vascular smooth muscle cells (VSMC) and vascularendothelial cells (VEC), esculetin (100 μM) was found to inhibit the formation of 12- and 15-HETE and decrease the productions to 23.0% and 37.7%, respectively. These indicate that esculetin is the most potent in blocking 12-LOX. The inhibitory potency on LOX is not related to the oxidation potential of the compound.

Esculin, glucosidation at one of the hydroxy group of esculetin, decreases the inhibitory potency markedly with IC$_{50}$ = 290 μM. 7-Hydroxy coumarin, a metabolite of coumarin, has the ability to reduce edema in the rat paw carrageenan test. It was also shown to inhibit rat platelet lipoxygenase (IC$_{50}$ = 502 μM) and prostaglandin synthesis. However, without hydroxyl in the position of C-6 and C-7, coumarin and 4-HC had no inhibitory effect on either enzyme at concentrations up to 1 mM. The effect of daphnetin and fraxetin on the formation of 5-HETE and the cyclooxygenase
product (12-hydroxy-5,8,10-heptadecatrienoic acid (HHT)) in polymorphonuclear leukocytes were studied. The results showed that daphnetin and fraxetin inhibit the formation of 5-HETE more strongly than HHT; the concentrations of IC$_{50}$ were, respectively, 6.90 mM, 2.57 mM and 139.0 mM, 532.5 mM. In addition, scopoletin (75) (Figure 19) were also show to inhibit the formation of 5-HETE and HHT, but less strongly. Therefore, that hydroxylation at C-6 is very important for esculetin to inhibit LOX. Scopoletin, derived from P. sabulosa,\textsuperscript{153} produced a dose related antinociception in the acetic acid-induced model of visceral pain in mice.

![Figure 19](image)

Two structural derivatives of scopoletin, acetylscopoletin (99) and benzoylscopoletin (100) (Figure 19) also exhibited antinociceptive activity. Benzoylscopoletin decreased the acetic acid-induced abdominal contraction 2-fold more than the original molecule. Structure activity relationship studies showed that the addition of a benzoyl group might increase the absorption of the compound or facilitate its binding to the active site in order to increase antinociceptive properties. The addition of an acetyl group, as in acetylscopoletin, decreases the anti-nociceptive properties of the compound by approximately 26-fold compared to the original coumarin. Sets of coumarinyl ethers having chromone, benzofuranyl and 4-hydroxy coumarins were prepared and tested for analgesic and anti-inflammatory activity. The results showed that these heterocyclic derivatives exhibited both anti-inflammatory and analgesic activity. The benzofuranyl ethers of coumarins were found to be most active amongst all the compounds. The chloro and methoxy substitution in coumarin
ring showed increase activity.\textsuperscript{154} Three major coumarins, edgeworin (EdN) (101),
edgeworosides A (EdeA) (102) and edgeworosides C (EdeC) (29) (Figure 20), isolated
from \textit{Edgeworthia chrysantha} L., were evaluated for both anti-inflammatory and
analgesic activities. The results showed that EdN and EdeA had anti-inflammatory ($p$
$< 0.05\text{-0.01}$) and analgesic ($p < 0.001$) effects, while EdeC showed only an analgesic
effect.\textsuperscript{149}

![Edgeworin (EdN), Edgeworosides A (EdeA), Edgeworosides C (EdeC)]

\textbf{Figure 20}

1.4.6. Anti HIV activity:

\textsuperscript{155} (+)-Calanolide A (103) (Figure 21), isolated from a tropical rainforest
plant of the species \textit{Calophyllum lanigerum}, was found to be active against HIV-1.\textsuperscript{156} (+)-
Calanolide A inhibits not only wild-type HIV-1 but also clinically isolated resistant
strains such as A17 (Y181C mutant). Studies (phase I) of single doses and multiple
escalating doses of (+)-calanolide A have demonstrated its safety and a
pharmacokinetic profile, which could significantly reduce viral load in HIV patients
with a dose of 600 mg (bid) in a monotherapeutic trial, worthy of further clinical
study.\textsuperscript{157-158}

Interestingly, (+)-Calanolide A is the first natural product identified as
active against HIV-1 and has recently been investigated continuously in phase II/III
clinical trials. The other (-)-Calanolide A (104-109) (Figure 21) also isolated from
\textit{Calophyllum lanigerum} were found to be HIV-1 reverse transcriptase inhibitors.\textsuperscript{159}
Since then, some other coumarin analogues, tetracyclic pyranocoumarins, the inophyllums isolated from the genus *Calophyllum ionophyllum* P (110-113) (Figure 21), were shown to be capable of inhibiting HIV-1 RT, though the most active inophyllums (110) and (111) have slightly different stereochemistry to the equally potent Calanolides.\(^{159}\) Structure activity relationship studies show that bulky substituents are required at C-4 position, both calanolides and inophyllums require methyl groups at C-10 and C-11 of the chromanol ring to be trans-diaxial, and both require a hydrogen bond acceptor at C-12. In case of calanolides, the C-12 hydroxyl should be S con\(\text{Figure}\), or carbonyl can be present. C-12 hydroxyl of inophyllums can be either S or R con\(\text{Figure}\), but cannot be a carbonyl.\(^{160}\)

![Chemical Structures](image)
1.4.7. Anticoagulant activity

Thrombosis is a major cause of morbidity and mortality in the industrial world. Intravascular thrombosis leads to myocardial infarction, stroke or pulmonary thromboembolism, which is one of the most common causes of death worldwide. For decades coumarins have been the most commonly prescribed drugs for therapy and prophylaxis of thromboembolic conditions. Warfarin (114) (Figure 22), oral anticoagulants of the 4-hydroxycoumarin class, represents the most commonly approved drug for therapy and prevention of thromboembolic conditions for over 50 years. While its market form is the racemic sodium salt, the anticoagulant activity of the (S) (−) enantiomer is known to be six times higher than that of the (+) enantiomer.161

Currently, the anticoagulant effect of warfarin is indirectly measured through the correlation of the clotting time (prothrombin time) and the amount of the drug present in blood.162

![Figure 22](image)

There is considerable difference in activity between the isomeric dicoumarol derivatives, 3, 3’-methylene bis (4-hydroxy-5, 7-dimethoxycoumarin (115) and 3, 3’-methylenebis (4-hydroxy-7,8-dimethoxycoumarin) (116) (Figure 22). The higher activity of the compound with substituents in position 8 showed the importance of its location.
Difenacoum 117 (Figure 23) is a synthetic derivative of Warfarin class of compounds. It is used as a rodenticide against pest rodents and acts by preventing the production of blood clotting factors.163

Dicoumarol 118 (Figure 23) is an anticoagulant that functions as a Vitamin K antagonist (similar to Warfarin). It is also used in biochemical experiments as an inhibitor of reductases.164 Bromadiolone 119 (Figure 23) is vitamin K antagonist, a second generation derivative of dicoumarol, with greater potency and longer activity.165

Robert et al synthesized a new series of 3-carboxamide-coumarins 121 (Figure 23) and their derivatives. These are the first potent and selective nonpeptidic inhibitors of FXIIa.166 Furthermore, the inhibitory effects of numerous coumarins, synthesized or isolated from plants, on anticoagulant activity have also been reported.167-173
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