Chapter 01

General Introduction
1.1 Introduction

Naturally occurring compounds are becoming of great importance due to their vast use in different sectors. Coumarins are such compounds which are in use since last few hundred years as a pure compound. Chemistry of Coumarin has taken an important place in recent years because of the discovery of the varied biochemical properties, industrial uses and analytical applications. A considerable amount of work has been done on coumarins and has been reviewed by a number of researchers. Coumarin is a compound which belongs to group of compounds called benzopyrones. There are 2 different types of benzopyrones namely Benzo-2-pyrones and Benzo-4-pyrones. Benzo-2-pyrones and Benzo-4-pyrones are known as Coumarins and Chromones respectively. Benzo-2-pyrones are commonly called as Coumarins, are a fascinating group of compounds occurring widely in nature, both in free and combined states.

Coumarin is used in the pharmaceutical industry as a precursor molecule in the synthesis of a number of synthetic anticoagulant dicoumarol, notably warfarin (which has a common and confusing brand name Coumadin) and some even more potent rodenticides that work by the same anticoagulant mechanism. Coumarin has clinical & medical value by itself, as an edema modifier i.e. anti-inflammatory activity. Coumarin and other Benzopyrones, such as 5,6-Benzopyrone, 1,2-Benzopyrone, Diosmin and others are known to stimulate macrophages to degrade extracellular albumin, allowing faster resorption of edematous fluids Coumarin is also used as a gain medium in some dye lasers. Properties, suggesting one reason for its widespread occurrence in plants, especially grasses and clovers are because of its effect of reducing the impact of grazing animals. Although the compound has a pleasant odor, it has a bitter taste and animals will avoid it. Recent studies indicate that the mediators and cellular effectors of inflammation are important constituents of the local environment of tumors. Inflammation in the body’s response to noxious or injurious stimuli, characterized by warmth, redness of the skin, pain, swelling and loss of function. It is a part of host defense mechanism. There are several tissue factors that are known to be
involved in the inflammatory reactions such as release of histamines, bradykinin and prostaglandins\(^2\). Coumarin has appetite-suppressing Inflammatory diseases are becoming common in aging society throughout the world.

Coumarins (2\(H\)-1-benzopyran-2-ones) are important oxygen containing fused heterocycles used in drugs and dyes\(^3\). The name of coumarine originates from ‘coumarou’ the vernacular name of the Tonka bean (Dipteryx odorata willd, Fabaceae), from which coumarin itself was isolated in 1820\(^4\). They are the family of lactones containing Benzopyrone skeletal framework that have enjoyed isolation from plant as well as total synthesis in the laboratory\(^5\). And immediately attracted the attention of perfumers on account of its pleasant and persistence odor. There are many number of naturally occurring and synthetic monomeric coumarin derivatives which are used in drugs and dyes\(^6\).

The incorporation group as a fused component into parent Coumarin alters the property of parent Coumarin and converts it into a more useful product\(^7\). Coumarin is plant flavonoids widely distributed in nature. Natural coumarins are known to have antidiabetic activity\(^8\), anabolic antioxidant and hepato protective activities\(^9\). Substituted Coumarins derivatives have been reported to have variety of biological activities. The potent antibiotics like Novobiocin, Coumaromycin and Chartesium are Coumarin derivatives. Recently, the interest on these compounds has been revived owing to their use as fluorescent markers in the biochemical determination of enzymes.

Coumarin derivatives can be synthesized by one of such methods as the Claisen rearrangement\(^10\), Perkin reaction\(^11\), Pechmann reaction\(^12\), Witting reaction\(^13\), as well as the Knoevnagel condensation\(^14\). Derivatives of Coumarins usually occur naturally as secondary metabolite present in seed, roots and leaves of many plant species\(^15\). These investigations have revealed their potentials as versatile biodynamic agent for example-3-heteroaryl substituted coumarin and Benzocoumarins of potential interest as pharmaceuticals and photochromic dyes\(^16\). Introduction of fluoro and sulfonamide moieties into Coumarin side chain hoping for an improvement of biological activity because, incorporation of fluorine to various heterocycles
is known to influence the biological activity\(^{17}\) and the sulfonamide moiety itself possesses important antibacterial\(^{18}\) and antitumor activity.\(^{19}\)

This large class of naturally occurring substances made of fused system of Benzene and one 1\(\alpha\)-pyrone ring, of which two distinct types are recognized as Benzo-\(\alpha\)-pyrones, commonly called Coumarins and Benzo-\(\gamma\)-pyrones, called Chromones, the latter differing from the former only in the position of the carbonyl group in the heterocyclic ring\(^{20}\).

![Benzo-a-pyrone Benzo-g-pyrone](image)

The researchers had shown on the basis of their investigation on Coumarin compounds that these compounds are promising candidates for different types of diseases due to their wide spectrum and diverse biological properties and various effects on the different cellular systems. Coumarins have important effects in plant biochemistry and physiology, acting as antioxidants, enzyme inhibitors and precursors of toxic substances. In addition, these compounds are involved in the actions of plant growth hormones and growth regulators, the control of respiration, photosynthesis, as well as defense against infection.

**1.1.1 Some Naturally occurring derivatives of Coumarins**

![Alloxanthoxyletin Paepalantine](image)
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The Coumarins as such are forming a large class of compounds which are extremely variable in structure, due to the various types of substitutions in their basic structure and hence can influence biological activity. For example, Carbochromen is a potent specific coronary vasodilator used for many years in the treatment of angina pectoris. A number of natural and synthetic Coumarin (2-Oxo-2H-chromene) derivatives have been reported for their notably possessing varied biological activities that can be briefly summarized as mentioned below:

- Anti-HIV
- Anticoagulant
- Antimicrobial
- Antifungal
- Anti-tuberculostatic
- Anti-cancer

Due to varied biological activity of Coumarins analogues, researchers are now taking interest and doing a lot of work on the biological properties of various synthetic and naturally occurring Coumarins. On a very concise note, this can be understood by the below mentioned discussion on pharmacological importance of this class of compounds.

1.2 Pharmacological importance

1.2.1 Coumarins as cytotoxic agents

Coumarins has attracted intense interest of the researchers in recent years because of their diverse pharmacological properties. The cytotoxic Coumarins can be a highly exploitable source of new anticancer agents, which might help the world in addressing cytotoxicity and resistance phenomena. These naturally occurring compounds have served as valuable leads for further design and synthesis of more active analogues. A very important and promising data have been reported by Raleva S.; Savov A.; Froloshka L.; Dundarova D.; Manolov I.; Argirova R for a series of different coumarins used as cytotoxic agents.

A varied number of structurally modified Coumarins’ derivatives have been reported to show substantial cytotoxic and anti-HIV activity during in vitro and in vivo studies. Coumarins have shown cytotoxicity with derivatives containing o-
Dihydroxy substituents as reported by Kolodziej et. al. The chemical structure and biological activity study of the Coumarins showed that the addition of a catecholic group to the basic structure enhanced cytotoxic activity in tumor cell lines.

1.2.2 Structures of cytotoxic Coumarins

- Coumarin
- 4-Hydroxycoumarin
- 6-Nitro-4-hydroxy coumarin
- 6,7-Dihydroxy coumarin (Esculetin)
- 7,8-Dihydroxycoumarin (Daphnetin)
- Coumarin-3-carboxylic acid
- 4-Methyl-6,7-dihydroxy coumarin
- 4-Methyl-7-hydroxy coumarin (Mendiaxon)
- 7,8-Dihydroxy -6-methoxy-2H-chromen-2-one
The Aminocoumarin antibiotics especially Novobiocin, Clorobiocin and Coumermycin A1 are known as potent inhibitors of gyrase. Their equilibrium dissociation constants are in the range of 10 nM, i.e., their affinity for gyrase is considerably higher than that of modern Fluoroquinolones. Novobiocin has been licensed as an antibiotic for clinical use (Albamycin; Pharmacia-Upjohn) and is used for the treatment of infections with multi resistant gram-positive bacteria, e.g. Staphylococcus aureus.

Novobiocin is produced by *Streptomyces spheroides* (syn. *S. caeruleus*) NCIMB 11891, Clorobiocin is produced by *S. roseochromogenes var. oscitans* DS12.976 and
coumermycin A1 is produced by *S. rishiriensis* DSM 40489.43 Obviously, these organisms must protect their gyrase from the inhibitory effect of Aminocoumarin during antibiotic formation.

Thiara and Cundliffe44-46 also reported that the principal resistance mechanism of the novobiocin producer *S. sphaeroides* is the *de novo* synthesis of a Coumarin-resistant gyrase B subunit, which replaces the sensitive GyrB subunit in the active (GyrA)2(GyrB)2 heterotetramer. Thus, this novobiocin producer contains two *gyrB* genes, a constitutively expressed *gyrBS*, encoding the Coumarin-sensitive protein and the *gyrBR* gene, encoding the resistant protein and expressed in the presence of novobiocin. The promoter of *gyrBR* appears to be regulated by changes in the
superhelical density of DNA. They used the Novobiocin producer *S. niveus*, which has recently been identified as a subjective synonym for *S. spheroids*.\(^4^7\)

### 1.2.3 Coumarins as potent anti-HIV compounds

Naturally occurring Coumarins and their derivatives can display an array of anti-HIV activity through different mechanisms that include blockade of viral entry, inhibition of reverse transcriptase and interference with viral integration\(^4^8\). Some of the Phenylcoumarins and Chalcones, as well as tannins and lignins, have been proposed as suppressors of LTR-dependent transcription, but the mechanism of action has not been fully understood characterised.\(^4^9\)

(+) -Calanolide A, a natural Dipyranocoumarin is currently undergoing anti-AIDS clinical trials, has also proven to be a very effective antimycobacterial against drug-resistant *Mycobacterium tuberculosis* strains.

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\text{\textit{\textbf{+}}-\textit{Calanolide A}}
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It has been reported by researchers that Mesuol and Isomesuol, the two 4-Phenylcoumarins, isolated from the tree *Marila pluricostata*, suppress *HIV*-1 replication in Jurkat T cells.\(^5^0\) These Coumarins do not affect the reverse transcription and integration steps of the viral cycle and their antiviral effect is additive with that of Azidothymidine (AZT). Alongwith the above mentioned activity, Mesuol also inhibits TNFα-induced *HIV*-1-LTR transcriptional activity by targeting the nuclear factor-κB (NF-κB) pathway. While mesuol does not prevent either the binding of NF-κB to DNA or the phosphorylation and degradation of NF-κB inhibitory protein, IκBα, it inhibits the phosphorylation and the transcriptional activity of the NF-κB p65 subunit in TNFα-stimulated cells. These results highlight the potential of the
NF-κB transcription factor as a target for anti-\textit{HIV}-1 compounds such as 4-Phenylcoumarins, which could serve as lead compounds for the development of additional therapeutic approaches against AIDS.

![Chemical structures of the 4-phenylcoumarins Isomesuol and Mesuol.](image)

### 1.2.4 Protease inhibitors

Warfarin is showing to inhibit the vit-K dependent conversion of Prothrombin and serine protease activity of thrombin. A 100 \( \mu \text{M} \) dose of Warfarin is inhibitory toward \textit{HIV} aspartyl protease. Warfarin shows the below mentioned four remarkable properties

1. Inhibition of serine protease,
2. Anti-Aspartyl protease,
3. Inhibitory activity for Reverse transcriptase and integrase,
4. All of which are essential for \textit{HIV} replication.

Therefore this drug deserves a thorough clinical testing in a larger population of \textit{HIV}-positive individuals for the betterment of society. Parke-Davis, a division of Warner Lambert and Pharmacia \& Upjohn, two big pharmaceutical companies, confirmed that Warfarin and related Coumarin compounds shows HIV protease inhibitory\textsuperscript{51,52} activity.
1.2.5 Integrase inhibitors

In addition to HIV RT and protease, HIV integrase is also a major chemotherapeutic target and integrase inhibitors mainly include Biscatechols and Coumarins. Increasing the number of aryl rings on the central linker enhanced potency the rigid Stilbene analogues. 7-Hydroxylation was beneficial in a wide range of dimeric-4,7-Hydroxycoumarins and led to a simplified coumarin integrase inhibitor without greatly sacrificing the potency of the tetrameric compound. A natural tetrameric coumarin showed high anti-integrase activity (IC$_{50}$ ¼ 0.8 µM for integration and 1.5 µM for 30-processing) (A Natural tetrameric coumarin).
1.2.6 Reverse transcriptase inhibitors

HIV-1 RT interacts with complementary Oligodeoxynucleotide (ODN) primers at the 50-end of the tRNA binding site as well as at the 30-end of the primer. ODNs conjugated to Chromone or Coumarin changed the polymerization rate: either inhibition or slight activation followed by inhibition depending on the concentration. When ‘‘chain terminator’’ 30ddT was added, the ligand-ODN complex was easily converted to a strong inhibitor.\textsuperscript{56}

Coumarin is simple oxygen containing heterocycle, benzopyran-2-one present in melitot, clover and tonca beans\textsuperscript{57} having extensive biological activities like antioxidant\textsuperscript{58}, analgesic\textsuperscript{59}, anti-inflammatory\textsuperscript{60}, antimutagenic\textsuperscript{61}, antibacterial–antifungal\textsuperscript{62}, anti HIV\textsuperscript{63} and mild adrenergic properties\textsuperscript{64}. The synthesis of Coumarins and their derivatives has attracted considerable attention from organic and medicinal chemists for many years as a large number of natural products contain Coumarin nucleus\textsuperscript{65}. They are widely used as additives in food, perfumes, cosmetics, pharmaceuticals and optical brighteners and dispersed fluorescent and laser dyes\textsuperscript{66}. Renewed attempts for the synthesis of Coumarins in an easiest and cheapest way have been progressing so as to meet the greater demand for this high potent pharmacologically active scaffold, by the conventional\textsuperscript{67-68} as well as modern methodologies\textsuperscript{69,70}.

The recent use of microwave technique in the preparation of Mannich bases has provided an elegant way to obtain these materials in a few minutes through an environmentally benign green chemistry approach in synthesis\textsuperscript{71}.
1.3 References


57. Clinton Salvador, Laskowski; *ibid.*, 1949, **72**, 3366.


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