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Chiral Anticoagulants Drugs Based on Coumarin

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Abstract:

Oral anticoagulants are widely used for treating and preventing thromboembolic diseases. The most common oral anticoagulatory agents are vitamin K antagonists which belong to the coumarin family, their target is to inhibit the enzyme vitamin K epoxide reductase, so they block the hepatic synthesis of the active, reduced form of vitamin K necessary for carboxylation of coagulation factors. Among these class of drugs acenocoumarol which is commercialized as Sintrom® is a chiral oral anticoagulant drug that belongs to the group of vitamin K antagonists, and it administered orally as a racemic mixture of R (+) and S (-) enantiomers due to its chemical structure that has one asymmetric carbon, there by its chiral propriety gives rise to different special arrangements and it can differ in potency, toxicity, and behavior in biological systems, and it have to be separated to investigate and study each single enantiomer.

Keywords: coumarin; anticoagulant; chiral, hemostatic, acenocoumarol; warfarin

1. Introduction:

Blood coagulation is a natural process in human body in case of injury and internal damage of vessels known as haemostasis, which contains a series of reactions involved platelets, proteins, vascular components, and specific enzymes to generate the thrombin and form the haemostatic plug in healthy persons. But in the case of abnormal blood coagulability which caused thrombus formation which breaks loose and is carried by the blood flow to plug in the body 's vessels system and it poses a dangerous risk on the lungs, heart, brain, and legs venous. Over the world the thromboembolism disease is a significant cause of morbidity and mortality, and oral anticoagulants are the drug of choice in many cases of prophylaxis and to reduce the thromboembolic disorder for long term treatment since the discover of dicoumarol by Carl Paul Link in 1940 and also known as vitamin K antagonists which including acenocoumarol, warfarin, phenprocoumon and related 4- hydroxycoumarin derivatives are commonly used in these clinical cases to avoid thrombus formation in case of VTE, PE, stroke and cardiovascular disease, these drugs are administered as racemate and produce their effect by inhibition of vitamin K-dependent γ-carboxylation of coagulation factors II, VII, IX, and X, which produce a biological inactive forms of these coagulation proteins, by the same manner the synthesis of the inhibitors proteins C and S is impaired. Chemically vitamin K antagonists have the same basic structure with stereogenic center and differs in substitutions groups which give different pharmacologic and pharmacodynamic proprieties to each drug. In this work we will cite the biological, chemical and chiral proprieties of oral anticoagulants coumarin based generally and acenocoumarol the subject of this study in detail which is prescribed in Algeria as Sintrom® 4 mg and minisintrom® 1 mg tablets.

2. Basic concepts of hemostasis:

Hemostasis is a natural process in human it controls circulation and fluidity of blood in through the body's vessels and arteries, it acts as balance between coagulant and anticoagulant factors. When a blood vessel is injured, the blood flows out until it will be stopped, hemostasis or the stopping of bleeding must be sharply distinguished from the blood clot formation, which is simply the gelification of liquid blood in vitro as a consequence of fibrin formation. Spontaneous hemostasis and blood clot formation are

are preceded by reactions referred to collectively as the hemostasis mechanism and the coagulation mechanism, respectively [4].

Hemostatic response can be conceptually divided into different interdependent phases: primary and secondary hemostasis as a purpose module. Primary hemostasis involves cellular components, conveniently divided into two groups: blood vessel sub-endothelial cells and blood platelets. The secondary phase involves the plasma coagulation proteins, the coagulation cascade. Primary and secondary hemostasis act synergistically, and it takes a few minutes to prevent excessive blood loss without starving the surrounding tissues of nutrients transported to them from blood [3]. Furthermore, this process involves the participation of the vessel wall, blood platelets, the coagulation system and, in a less defined way, the fibrinolytic enzyme system [5].

2.1 Primary hemostasis:

The primary hemostasis is beginning after endothelial damage, comprises the process of platelet adhesion, activation and aggregation, to form a platelet plug at the site of injury**,** otherwise this process encompasses all aspects of platelet adhesion and aggregation. Apart from platelets, components of the vessel wall sub-endothelial matrix components in particular and Von Willebrand factor (VWF) are involved in this process [6,9].

2.1.1. Platelet:

Platelets are the primordial players in primary hemostasis. There are normally between 150 and 400 billion platelets per liter of blood in a healthy adult, produced by megakaryocytes in the bone marrow [8,10]. Platelets are disc-shaped, anuclear cells that contain a contractile system, storage granules, and cell surface receptors. Platelets normally circulate in a non-activated state in the blood but are extremely reactive to changes in their environment. Platelet membranes contain receptors for a variety of agonists including ADP, thromboxane A2, and platelet activating factor, immune complexes, and thrombin. Serotonin and epinephrine synergistically promote aggregation induced by other agents [11].

2.1.2. Structure and function of platelet:

On activation, platelets change from the normal disc-shape to a compact sphere with long dendritic extensions facilitating adhesion (FigureI.1). The cytoplasm is rich in actin and myosin which bring about the change in shape and retraction of the clot. There are two classes of secretorygranules.

The first type are dense granules that secrete ADP and calcium, which reinforce platelet aggregation and platelet-surface coagulation reactions. The second type are granules, which secrete a vast array of proteins: some, such as von Willebrand factor and platelet factor 4, aresynthesised by megakaryocytes; others, such as fibrinogen, are acquired from the plasma by receptor-mediated endocytosis; still others, such as the abundant plasma proteins, albumin and IgG, are acquired by fluid-phase pinocytosis [13].

As a result of primary hemostasis, a primary platelet plugs forms on the injured endothelium, mainly consisting of platelets and VWF. This platelet clot is further modified and stabilized by crosslinking of fibrin [12].

2.1.3. The endothelium (vessel's wall):

The walls of vessels differ based on whether it is carrying blood from or towards the heart. Arteries are exposed to a much higher internal pressure and so are thicker andmorphologically more complex than veins, which carry blood from tissues to the heart at a lower pressure [14].

The endothelium plays a crucial role in providing the proper hemostatic balance. The function of endothelial cells far exceeds that of providing a non-thrombogenic inner layer of the vascular wall that helps to maintain blood fluidity. Under physiological conditions, endothelial cells prevent thrombosis by means of different anticoagulant and antiplatelet mechanisms. These cells are involved in all main hemostatic pathways triggered upon vascular injury and limit clot formation to the areas where hemostasis is needed to restore vascular integrity [15].

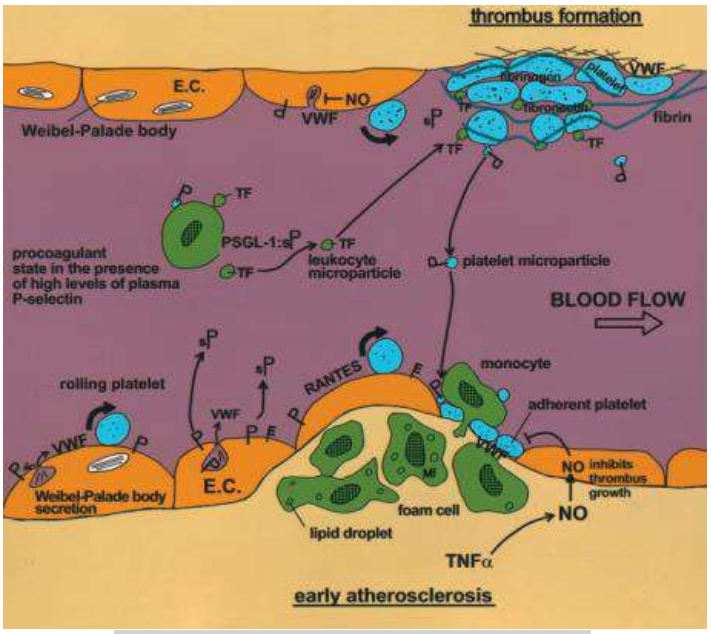


Figure 1: structure and function of platelets.

2.1.4. Endothelium morphology and endothelial cell function:

The endothelial cell (EC) surface in an adult human is composed of approximately 1 to 6*1013 cells, weighs approximately 1 kg, and regulate the flow of nutrient substances, diverse biologically active molecules, and the blood cells themselves. This gate-keeping role of endothelium is effected through the presence of membrane-bound receptors for numerous molecules including proteins (eg, growth factors, coagulant, and anticoagulant proteins), lipid transporting particle (eg, low-density lipoprotein [LDL]), metabolites (eg, nitrous oxide and serotonin), and hormones (eg, endothelin-1), as well as through specific functional proteins and receptors that govern cell-cell and cell-matrix interactions **[**16].

Not only do they form the structural basis of blood vessels and provide an anti-thrombogenic surface, but they also contribute to numerous metabolic functions including coagulation and

thrombolysis, control of vasotonus and antigen presentation, as well as basement membrane and growth factor synthesis. Endothelial cell adhesion molecules regulate the trafficking of circulating cells and provide, thus, an internal map that regulates body compartmentation [17].

2.2. Secondary hemostasis:

Secondary hemostasis relies on coagulation factor activation which generates insoluble fibrin fibres. The 1964, McFarlane's 'Coagulation Cascade' divided the process of coagulation into three distinct parts, namely the intrinsic, extrinsic and common pathways, suggesting that both the intrinsic and extrinsic pathways were independently capable to initiating clot formation [18].

3. Coagulation system modeling:

There are two models described, the complicate process of coagulation during hemostasis the
cascade hypothesis [22], and the cell-based model [20]. Coagulation system is a very complicate physiological, autocatalytic process, and requires a straight regulation. The fundamental role of the coagulation system which provides for immediate activation when there is blood loss that needs to be stemmed but also confines its activity to the site of blood loss. Otherwise, coagulation might occur throughout the entire circulatory system, which would be incompatible with life and some of the processes controlled by enzyme inhibition are blood coagulation (hemostasis), blood clot dissolution (fibrinolysis), complement activation, connective tissue turnover, and inflammatory reactions [1-3].

3.1. Model of the coagulation cascade (the waterfall model):

The hypothesis of the coagulation cascade consists of two pathways the intrinsic and the extrinsic (FigureI.2), The sequence initiated by collagen is called the intrinsic pathway and involves components normally present in circulation. The extrinsic pathway is so called because it involves tissue factors as well as blood components. The two pathways share a common terminal sequence from factor X to the formation of fibrin [21,29].

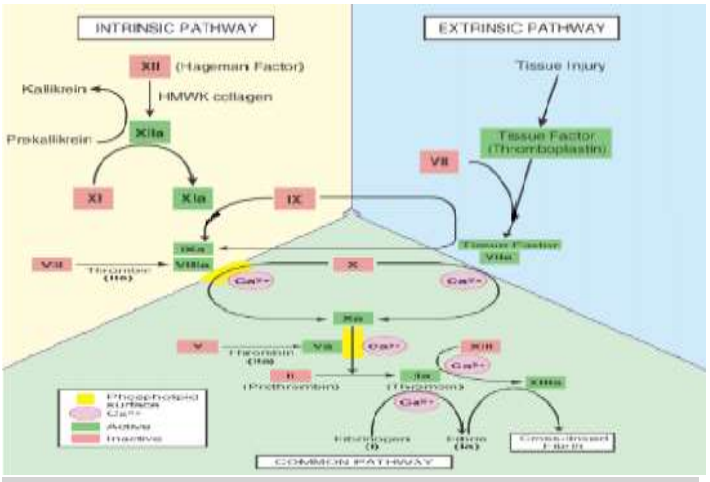


Figure 2: the extrinsic and intrinsic pathway of coagulation cascade model.

3.2. The cell-based model:

We view hemostasis as occurring in three (overlapping) phases (Figure I.3). The initiation of coagulation takes place on TF-bearing cells, such as the fibroblast. If the pro-coagulant stimulus is sufficiently strong, enough factors Xa, IXa and thrombin are formed to successfully initiate the coagulation process. Amplification of the coagulant response occurs as the "action" moves from the TF-bearing cell to the platelet surface. The pro-coagulant stimulus is amplified as platelets adhere, are activated and accumulate activated cofactors on their surfaces. Finally, in the propagation phase, the active proteases combine with their cofactors on the platelet surface, the site best adapted to generate hemostatic amounts of thrombin. The activity of the pro-coagulant complexes produces the burst of thrombin generation that results in fibrin polymerization [22].

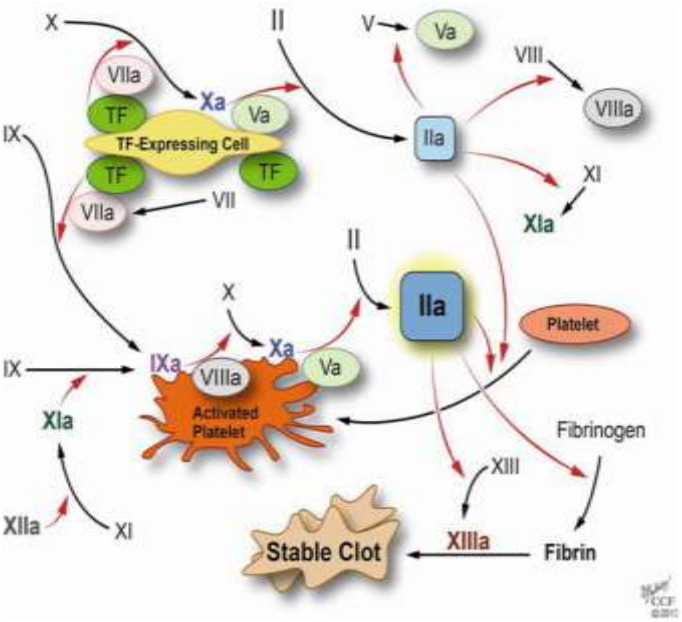


Figure 3: cell-based model of coagulation.

The pathophysiology of thrombus and embolus formation:

The English word "embolus" derives from a Greek word meaning "plug" or "stopper" Dr.McKee reported that the genesis and difference between thrombosis and embolism, however had not clearly defined. In thrombosis the clot may be found in anywhere and in the stationary case [31], In other hand Dr. Chandler propose that there may be more than one wayan embolus forms. One way an embolism forms is by breaking off from a mural thrombus. Another way is that an embolic thrombus might form in the bloodstream itself, in the flowing blood without first having formed on the wall and being dislodged. This can be nicely demonstrated experimentally and may indeed be relevant to some of these in vitro techniques [32].
The hypothesis sad that in slow-flow systems such as the leg veins or fibrillating atria, the fibrin network is often fine and randomly arranged, whereas in fast-flow major arteries the fibrin is coarse

and laminated, forming the 'Lines of Zahn'. The resemblance between hemostatic plugs and thrombi led to the belief that thrombosis was due to inappropriate clotting, triggered by the release of thromboplastins from damaged vessel walls, thereby activating the extrinsic pathway, or activation of the intrinsic pathway by contact between clotting proteins and vessel walls which had lost their protective endothelium and which were therefore providing the equivalent of an unnatural surface [30].

Moreover, thrombus formation (Figure I.4) is a natural process in hemostasis under normal circumstances when there is an injury in the vessel wall, it involves activation of platelets, activation of the coagulation system, and the processes of fibrin dissolution[24]; the first discover of the pathogenesis of the thrombolytic disorder was announced by Rudolf Virchow in his triad which has demonstrated that there are three possible contributors to the formation of an abnormal clot (thrombus): vessel wall injury or inflammation, changes in the intrinsic properties of blood, and decrease in blood flow velocity atherosclerotic plaques [23], which are found in most major arteries, are the main substrate for thrombus formation. Other factors that affect thrombus formation include the degree of plaque disruption and the content of tissue factor in the plaque. Stenotic arteries and blood velocity also affect the platelet disposition and thrombus formation as they change the shear rate of flowing blood [23,25].

By the years, other models of thrombus formation are described in vivo [26], to understand more and more the complex mechanism of thrombus formation, Sharlene M. Day et all[27] reported that the tissue factor which is a membrane bound protein is the key of initiation of the blood clot in the case of injury of a blood vessel, the TF forms a complex with the proconvertin factor (factor VII) produced in the liver to produce a clot in the site injury, Extension of the thrombus into the blood vessel lumen can obstruct blood flow, causing unstable angina and acute myocardial infarction. The TF/factor VIIa complex also plays a major role in the pathogenesis of venous thrombosis, a leading cause of morbidity and mortality.

The recent studies also reported the main contribution of platelets in the thrombus formation which can cause a serious danger in case of an abnormal disorder, Recent advances in intravitalmicroscopy have highlighted the dynamic nature of platelet thrombus growth in vivo.

In particular, they have established that the majority of platelets tethering to the injured vessel wall or to the surface of thrombi translocate for a variable period prior to forming firm adhesion contacts or detaching back into bulk flow. This phenomenon appears to be a general feature of thrombus formation, occurring in the arterial and venous circulation [28].

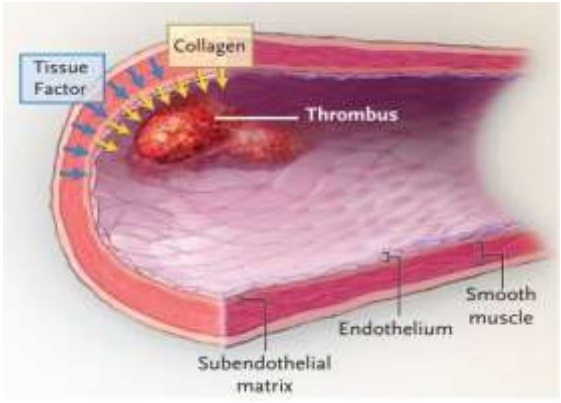


Figure 4: thrombus formation.

5. Causes of the risk of thrombus formation:

Certain systemic risk factors are also associated with thrombus formation, for example,lipoprotein has a similar structure to plasminogen, which may impair thrombolysis. Increased blood thrombogenicity is also associated with increased lowdensity lipoprotein (LDL). Poorly controlled diabetes mellitus results in glycosylation of collagen and protein, increasing the levels of plasma fibrinogen. Furthermore, smoking has been found to increase tissue factor levels in thrombotic plaques [25].

6. Thromboembolic diseases:

In this stage we must mentioned that aclotis morphologically homogeneous, dark-red structure that results from the coagulation of whole blood and is usually associated with venous circulation. The structural integrity of the clot is maintained by a fibrin network and the principal event in clot formation is the activation of fibrinogen to fibrin. It contains red cells, white cells and platelet s in roughly the same proportions as they occur in whole blood. Thrombi are simpler, more highly organized structures that occur with varying degrees of complexity, but the usual form on the arterial side of the circulation is a white head composed almost entirely of aggregated platelets , to which is attached a red tail of fibrin containing red cells . The principal events in thrombus formation are initial adherence and the propagation of platelet aggregation by ADP resulting from the platelet release reaction [33-34].

6.1. Venous Thromboembolism:

Venous thromboembolism (VTE), which comprises deep vein thrombosis (DVT) and pulmonary embolism (PE), which represent two clinical manifestation of the same disease, (VTE) has a clinical incidence of approximately 1−1.5 per 1000 annually in adult populations, about two-thirds of VTE episodes manifest as DVT and one-third as PE. Thrombogenesis generally occurs in the deep veins of the lower extremity. Pieces of thrombus can break free from the primary thrombotic lesion and embolize to the right heart via the inferior vena cava. The embolus can then become lodged in the pulmonary vasculature leading to the clinical diagnosis of pulmonary embolism [35-37].

6.2. Deep vein thrombosis (DVT):

DVT is a process of the blood coagulation in the deep vein axel, which is followed by a phlebitis inflammatory reaction in the leg veins. Practically it is the activation of coagulation in veins in areas of reduced blood flow, the pathogenesis of DVT is a complex and multifactorial event. Many diverse factors have been associated with DVT: immobilization, obesity, advanced age, oral contraceptives,trauma, surgery, cancer, heart disease and stroke.VTE is the third mo common cardiovascular disease after ischemic heart disease and stroke, the release reaction of platelets, the slowing of the blood and its eddying in the valve pockets of the leg veins often lead to the initiation of thrombi at these sites; with their formation there is further retardation of blood flow leading to propagation of the thrombus in both directions but chiefly in the direction of blood flow. These are the 'red thrombi'

of the deep leg veins which may be loosely attached and easily freed to cause emboli to the pulmonary arteries, producing sudden shock and possibly death [38-40].

6.3. Pulmonary embolism:

The deep veins network of the lower limbs is classically divided into two regions: the proximal and the distal territory. Noteworthy, proximal DVT is more frequently associated with pulmonary embolism (PE), and recurrencethan isolated distal DVT [44,83]. In other hand the clinical spectrum and pathophysiologic manifestations of PE range from small, incidental thrombosis to massive PE associated with sudden death due to cardiogenic shock, these can be distinguished on the clinical history and are (1) Acute minor embolism (2) Acute massive embolism (3) Sub-acute massive embolismand (4) Chronic thrombo-embolic pulmonary hypertension. Pulmonary arterial obstruction and platelet secretion of vasoactive agents elevate pulmonary vascular resistance. Increased alveolar dead space impairs gas exchange, and stimulation of irritant receptors causes alveolar hyperventilation**.** PE ranking as the third most common acute cardiovascular disease. Nearly 10,000 deaths were the result of PE or DVT in 2009 with PE having an estimated mortality rate of nearly 30%.DVT and PE account for more than 500,000 hospitalizations in the adult population and carry a large economic burden with a health care cost up to $33,200 per patient annually [41-43]

6.4. Paradoxical embolism:

In this rare and interesting phenomenon, a thrombus, detaching from a vein produces embolism in the brain, kidney, spleen, or other organ of the systemic arterial circulation. A communication between the right and left sides of the heart is essential, and in most cases, this is a patent foramen ovale, less often another septal defect [45].

7. Regime of treatment against thromboembolic disorder:

Thromboembolic disorder is a silent disease, which causes mortality in general cases, its diagnosis and treatment is complicate and requires a vigorous control. thus the anticoagulation therapy is required in several clinical condition it used in the prevention of cardioembolic stroke in the presence of atrial firillation (AF) or cardiomyopathy, cardioversion for arrhythmias such as atrial fibrillation, valvular heart disease (patients suffering mitral stenosis or regurgitation who have AF or a history of systemic embolism, left atrial thrombus, or an enlarged left atrium), mechanical prosthetic heart valves, acute coronary syndromes and ST-elevation myocardial infarction in the acute phase, bioprosthetic heart valves as reported by Keelig et al**[**35,49-50,52-54]; for the treatment of VTE the anticoagulation remains the mainstay since the landmark study by Barritt and Jordan more than 50 years ago reporting a significant reduction in mortality and recurrences in patients with symptomatic PE treated with intravenous unfractionated heparin (UFH) and oral anticoagulants[48].The first controlled trial of an oral anticoagulant in the prevention of venous thromboembolism was reported by Sevitt and Gallagher in 1959 [46]; currentlyavailable options for anticoagulation include treatment with unfractionated heparin (UFH), low-molecular-weight heparin (LMWH), vitamin K antagonists (VKAs), and, more recently, the synthetic penta saccharide fondaparinux. Although these drugs have proved effective in treating and reducing the risk of thromboembolic disease and it can be divided into three families [47,55].

7.1. Direct acting anticoagulants drugs:

7.1.1. Unfractionated Heparin and Low Molecular Weight Heparin:

Heparin is used on different forms therapeutic, it's the anticoagulant of choice when a rapid coagulant effect is required because its onset of action is immediate when administered by IV injection [51,57]. Heparin is not a discrete chemical entity, but rather a heterogeneous mixture of molecules ranging in molecular weight from around 4,000 to greater than 30,000. All molecules of heparin do not appear to have anticoagulant activity but only one third contain the high affinity pentasaccharide required for anticoagulant activity [56,65].

Clinically, unfractionated and low-molecular-weight heparins have been in use for more than 70 years while low-molecular-weight heparins have been important therapeutics for close to 30 years.

New generations of ultra-low molecular weight heparin are now in clinical trials, the administration of heparins require a strict laboratory control [60, 64].

7.1.2. Unfractionated heparin (UFH):

Unfractionated heparin is a group of molecules ranging in molecular weight from 3,000 to 30,000 kDa. Only about one-third of these molecules contain the requisite AT III-binding pentasaccharide sequence. Within this fraction, smaller heparin molecules containing fewer than 18 saccharide units (roughly 6,000 kDa) are not sufficiently long to mediate ATIII binding to thrombin but can still catalyze AT III/factor Xa inactivation [58].

7.1.3. Low molecular weight heparins LMWHs:

LMWHs are derived from UFH by chemical or enzymatic depolymerization to yield fragments
that are approximately one third the size of heparin [61,86]. LMWHs have smaller polysaccharide chains with a range of 1,000 to 10,000 (mean molecular weight of 5,000), the bioavailability of LMWHs following subcutaneous injection approaches 100 %. Peak anti-Factor-Xa activity occurs about 3–4 h following a subcutaneous dose [62-63].

7.2. Indirect acting anticoagulants drugs:

In this part of work, we are focus on the chemistry and pharmacology of the vitamin K antagonists which belong to the coumarin family and their derivatives. Coumarin are the most frequently used oral anticoagulation medications for prevention and therapy of thromboembolic conditions for long-term treatment [66-67].

8. Coumarin as oral anticoagulant drugs:

8.1. History of coumarin:

Coumarins are a large family of benzopyrones (1,2-benzopyrones or 2H-1-benzopyran-2-ones) widely distributed in the nature, the first isolation of coumarin as natural compound was in 1821s from the Tonka bean, which take its name from the French term ”coumaru” of this plant, chemically the basic structure of coumarin is an oxygen-containing heterocycles, bearing a typical benzopyrone framework, this compound and a great range of its structural derivatives have been shown to occur in hundreds of plant species, as well as in certain other organisms[70-73]. In 1933 a farmer in Wisconsin visited the chemical laboratory of Karl Paul Link because his cattle died of a mysterious hemorrhagic disorder at the end of the winter season. Link discovered that ingestion of spoiled sweet clover which caused a hypocoaguable state that increased the risk of bleeding, and in 1940 Link isolated successfully dicoumarol (3,3 ′-methylenebis-(4-hydroxycoumarin) the first oral anticoagulant [88], which is a coumarin oxidized and coupled with formaldehyde during sweet clover spoiling,vitamin K antagonists were originally used as a very effective rodent. After this innovation the Wisconsin Alumni Research Foundation in 1948 developed the new synthetic coumarin warfarin according to the abbreviation of this foundation WARF and “arin” according to coumarin, several anticoagulants were developed in Europ as acenocoumarol and phenprocomon [68-69,71-74].

8.2. Pharmacology of vitamin K antagonist drugs:

Clinically coumarins anticoagulant drugs are prescribed for different indications such as longterm treatment venous thromboembolism or prevention of systemic embolism or stroke in patients with prosthetic heart valves or atrial fibrillation [80,85-87].

8.3 Mechanism of action of oral anticoagulant drugs coumarin based:

The coumarins act as competitive inhibitors of vitamin K epoxide reductase, which is a cofactor for the post-translational carboxylation of glutamate residues to γ-carboxyglutamates on the Nterminal regions of vitamin K dependent proteins, and they have been shown to be effective in the prevention of thromboembolic complications in distinct clinical situations, which are responsible for regenerating reduced vitamin K from vitamin K epoxide, after it has been consumed as a co-factor in the synthesis of coagulation factors II, VII, IX, and X. This reaction presents a main role during the normal activation of clotting factors in the coagulation cascade (Figure I.5) [59,75-76,81-84,129].

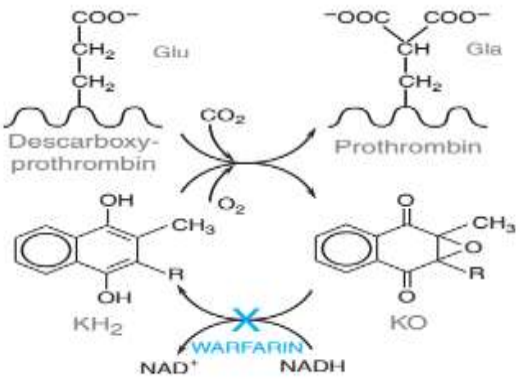


Figure 5: mechanism of action of an oral anticoagulant eg. Warfarin [84]

8.4. Advances in oral anticoagulant coumarin based coumarin therapy:

The vitamin k antagonists are the drugs of choice for many years before the development of the new oral anticoagulants, Therapy with coumarin derivatives is most effective when the international normalized ratio (INR) is kept within a narrow range [130,135], Acutely, many patients can be treated with parenteral anticoagulants, most commonly subcutaneous low molecular weight heparin, but oral anticoagulants are needed for long term treatment with VTE,AF and patients with heart prosthetic heart valve, the difficulty is the initial response to vitamin K antagonists (VKAs) which depends on the characteristics of the patient, including: age, sex, height, weight, concomitant drugs; dietary vitamin K intake, smoking status, alcohol intake, and genetic factors, which are the major determinant of oral anticoagulants drugs dose that’s because the single nucleotide polymorphisms (SNPs) in the genes that encode vitamin K epoxide reductase complex subunit 1 (VKORC1) and cytochrome P450 2C9 (CYP2C9) contribute largely to inter-individual variations in VKAs dose requirements, for this reason researchers have evaluated a pharmacogenetic algorithm for acenocoumarol and warfarin to determine dose requirement to the factor of variability [131-136].

However, only a limited number of studies are available to predict starting dose of acenocoumarol. The developed algorithms were population specific and cannot be used for any other population [137].

9. Chemistry and chiral proprieties of acenocoumarol:

9.1. synthesis and structure identification:

The synthesis of oral anticoagulants based on coumarin and their derivatives was widely reported in the literature; since the discover and identification of dicoumarol which used as a clinical anticoagulant[94], more than one hundred synthetic derivatives based on 4-hydroxycoumarine have been tested for their anticoagulant activity and this study was focus on the substitution of 4-hydroxycoumarin (Figure I.6) in position 3 and 4 with a keto group in position 5 as reported by Link and coworkers[96] also the presence of halogen atom can increase the anticoagulant ability of the molecule [89-90] other researchers reported that the modification on the structure of 4-hydroxycoumarin in position 8 and the alkylation in position C4 by condensation can also lead to

new molecules with an anticoagulant activity[91-93,101], other hypothesis was based on the studies of the structure of vitamin K to find the adequate structure of coumarin antagonist[97]. Recently researchers have synthesized successfully and tested the ability of anticoagulation of other several derivatives based on coumarin and mainly from 4-hydroxycoumarine, warfarin and phenprocoumon [92-95].

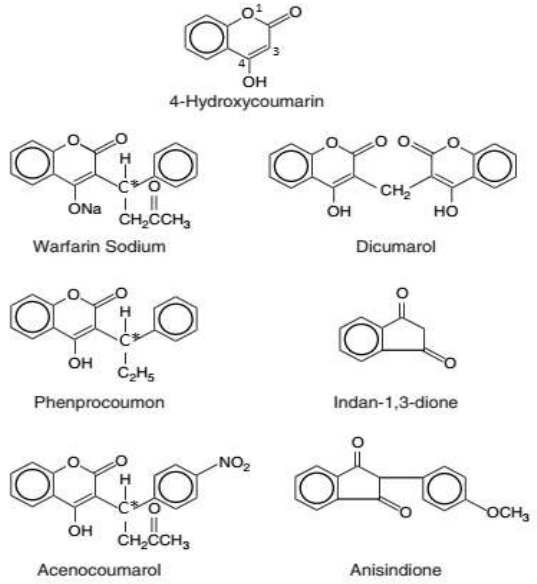


Figure 6: structure of the most common oral anticoagulants derivate from 4-hydroxycoumarin.

10. Objective:

This study is focus on the oral anticoagulant 4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2H chromen-2 one known as acenocoumarol, is one of the common anticoagulant of class 4-hydroxycoumarin administered to manage the thromboembolic disorder and administered as racemat orally quarter-scored film coated tablets, commonly known by the brand name Nicoumalone®, Sintrom® 4 mg and Minisintrom® 1 mg with the shorter half-life (from 8 to 24 h) Acenocoumarol therapy is challenging because patients exhibit a large variability in their anticoagulant response. This drug has a narrow therapeutic range, and small dose variations may result in hemorrhagic or thrombotic complications [98-100].

11. Acenocoumarol:

11.1. Chemistry:

11.1.1. Molecular structure and spectral identification:

Acenocoumarol is a synthetic oral anticoagulant of the coumarin type and mainly 4-hydroxycoumarin subclass, chemically acenocoumarol composed from 2H-1-benzopyrane nucleus (2H-chromene) substituted on position C4 by a hydroxyl group and an alkyl side chain in position C3 contains an other side chain with benzene cyclical nucleus substituted by nitro group in position 4($-NO2-$) and keto carbonyl group in position 3 on the main side chain (Figure I.7) [102-103].

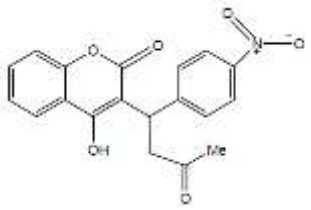


Figure 7: structure of acenocoumarol.

11.1.2. Synthesis:

The discovery and evaluation of coumarin anticoagulant action presented a main step in the treatment and prevention of thromboembolic disorder. In 1939 Karl Paul Link has isolated successfully the first anticoagulant dicoumarol, and after few years of experiences and in vivo tests, dicoumarol was introduced to clinical usage in 1942, the scientists were worked hardly to evaluate new series of AVKs more potent and have an effective action with short half-life, thus in 1955 Stoll and Litvan produced for the first time acenocoumarol (Sintrom, formerly G-23350) further to a series of substitution on warfarin [104-109].

11.1.2. a. Synthesis of Stoll and Litvan 1951:

In a first paper, Stoll and Litvan have reported that all compounds which have 3-(α-phenyl-β- acetylethyl)-4-hydroxycoumarin and 3-(α-phenyl-β-benzoylethyl)-4-hydroxycoumarin and corresponding compounds with methoxy or hydroxy group in the phenyl radical have an action on the coagulation process, and they have observed also that compounds of the general formula (figure I.8) in which R is a small hydrocarbon group or phenyl, halogenophenyl, metoxyphenyl or nitrophenyl radical, R1 represents halogen or nitro group and R2 is either a hydrogen, halogen or methyl group.

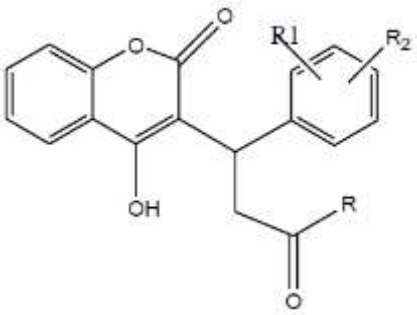


Figure 8: general structure formula of OA derivate from 4-hydroxycoumarin and α-β unsaturated ketones

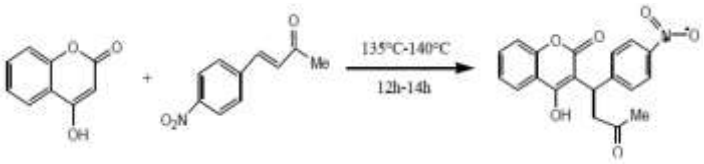
Procedure:

The general procedure to prepare acenocoumarol is done by addition reaction of 4-hydroxycoumarin with α-β unsaturated ketones of formula (Ph-CH=CH-CO-CH3) the reaction can be progress with basic condensation agents such as methanol, ethanol, water, pyridine or alkali alcholate and also in the presence of heating for several hours at 120°C to about 150°C.

Preparation:

16 parts of 4-hydroxycoumarin are mixed with 19 parts of 4-nitrobenzalacetone and heated at 135 to 140°C for 12-14 hours in

oil bath. After cooling, the melt was dissolved in acetone, and the product is collected; MP:196-199°C. (Scheme I.1)



Scheme 1: reaction of synthesis of acenocoumarol.

11.1.2.b. Manolov and co-workers:

Acenocoumarol was prepared by Michael addition of 4-hydroxycoumarin to 4-nitrophenyl-3-buten-2-one in the presence or absence of some basic and alkali catalysts giving a high yield between 46% and 92%

Preparation:

To an equimolar mixture of 4-hydroxycoumarin (50ml) and 4-nitro-3-buten-2-one (50ml) a 160 ml of water and the catalyst as described in the table below, were add under refluxing stirring after that the product is filtered, collected and washed with 300 ml of heated water, and with 100 ml of ether after cooling, the product is recrystallized from glacial acetic acid.

Duration (h)	Catalyst	Yield (%)
6	Without catalyst	78
3	Triethylbenzylammonium chlorid (5 mol %)	88
2	Benzylammonium chlorid (5 mol %)	92

Table 1: reaction of synthesis of acenocoumarol.

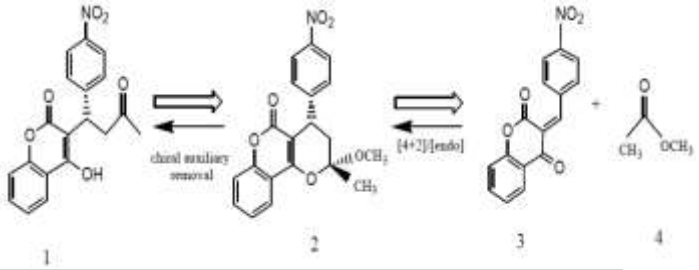
11.1.2.c. Cravotto and co-workers:

Hetero-diels-Alder cycloaddition:

Nowadays, the industry of active ingredients is focus on the synthesis of enantiopure molecules either by using large range of catalysts like Lewis acid's, enzymes, amino acids, chiral organocatlysis, or alkynes [110].

Procedure:

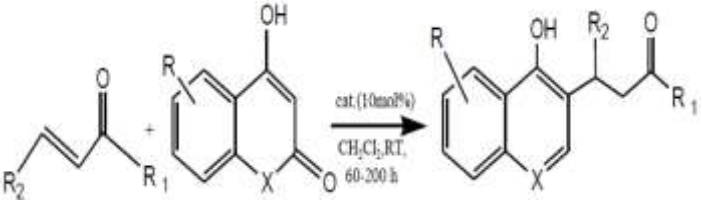
This reaction is based on thermal intermolecular HAD reaction, thus after the formation of the chiral auxiliary and its reaction in the situ generated the product in the presence of Lewis acid's. in this experiment the researchers use (1.1 equivalent) of 4 with 4-nitrobenzaldehyde (1.2 equivalent) and 4-hydroxycoumarin in dry dioxane in the presence of Tietz base and 5A° molecular sieves at 80°C during 10h after the isolation of the product and identified with spectrometric analysis (NMR, MS, TLC, HPLC); 4-nitrobenzaldehyd lead to (S)-acenocoumarol with 95% ee in 59% yield (SchemeI.2) [111].



Scheme 2: formation of acenocoumarol by HAD reaction.

11.1.2. d. Jorgensen and co-worker's synthesis:

In 2003, Jørgensen et al developed widely applicable imidazoline catalyst for one pot synthesis of anticoagulants generated from the reaction of 4-hydroxycoumarin compound and α-β unsaturated ketones by new organocatalyst reaction, they attempted enantioselective 1,4-Michael addition by using well known benzylideneacetophenone (Scheme I.3) [139].



Scheme 3: formation of acenocoumarol by imidazoline catalyst.

Procedure:

The reaction mixture was treated with (4S,5S) -4,5- diphenyl-imidazolidine-2-carboxylic acid (10mol%) in CH2Cl2 at room temperature for 130h to give 83% e.e (S)-acenocoumarol with 81% yield [112-113].

11.1.3. Spectral identification and physical-chemical proprieties:

Acenocoumarol (C19H15NO6) is a white, crystalline powder, tasteless and odorless, slightly soluble in organic solvents and water, its melting point reaches 197°C [114]. The structure of acenocoumarol has been assigned by CD, IR, 1H NMR and MS analysis, all data are illustrated in the table below [115-117].

IR data (cm^{-1})							
ν_{OH}	$\nu_{C=O}$ (a)	$\nu_{C=O}$ (b)	$\nu_{C=C}$	ν_{NO_2}	ν_{C-C}	ν_{C-O}	ν_{C-O-C}
3295	1686	1619	1572	1512	1294-1212	1173	1101
H NMR data δ (ppm)							
H6-H8 (c)		H9 (c)	H10 (c)	H12 (c)		H14,15,16 (c)	
7,36-7,66 (m)		4,18 s	3,47	1,94		7,83-8,12 (dd)	
MS data (%)							
M/z						(%)	
354						100	
295						17	
163						27	

Table 2: some spectral data of acenocoumarol.

a: keto carbonyl group. b: lactone carbonyl group. c: atom numbering is in agreement with the
structure in (figure I. 9)

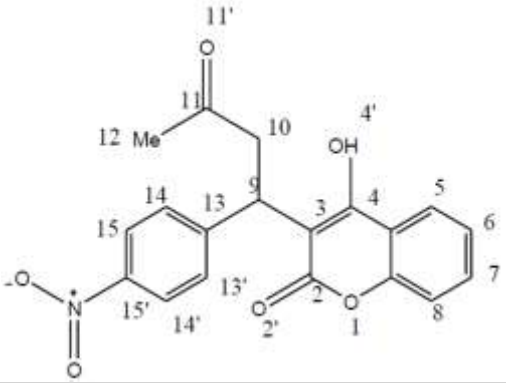


Figure 9: structure and atom numbering of acenocoumarol.

11.2. Pharmacodynamic and pharmacokinetics of acenocoumarol:

Acenocoumarol is the drug of choice in many cases of thromboembolism prophylaxis and treatment according to the British Approved Name, like the other single ring coumarin derivatives, warfarin and phenprocoumon, it contains an asymmetric carbon atom and thus exists as two enantiomers. Commercial acenocoumarol is a racemic mixture consisting of equal parts of R(+) and S(-) enantiomers**[**118-119,122]. For the mono-coumarin derivatives warfarin and phenprocoumon, it was shown that bouth in man and rats the S(-) enantiomer is more potent as anticoagulant than the R(+) enantiomer. And thus the differences in the affinity for the receptor site rather than differences in the pharmacokinetics due to these stereoselective differences in the anticoagulant potency, unlike warfarin, acenocoumarol differs only by a nitro-group in para position of the phenyl ring, wherein the in vitro pharmacological activities of R(+)acenocoumarol was more potent as anticoagulant and it is mainly the responsible for the overall anticoagulant effect, than S(-) acenocoumarol which has the faster elimination[120-121].

11.3. Metabolism, absorption and elimination:

In man the S-enantiomers of both compounds' warfarin and acenocoumarol are eliminated more rapidly than the R-enantiomers; the differences are modest for the warfarin enantiomers but 10-20 fold for the enantiomers of acenocoumarol. The body clearance of the acenocoumarol enantiomers is at least 100 times that of R/S-warfarin, wherein R -acenocoumarol has an elimination half-life of 9 h; while the S acenocoumarol has a plasma half-life <2h reach 0.5h. Approximately 98–99% of the coumarin anticoagulants are bound to plasma albumin, the metabolism into inactive metabolites takes place in the liver by various hydroxylation reactions, catalyzed by cytochrome P450 (CYP) CYP2C19 and CYP2C9 enzymes, 80% to 100% of R-acenocoumarol is primarily metabolized by CYP2C9 to 7-hydroxylation and 40% to 50% to the 6-hydroxylation with CYP1A2; and it is more potent than S-acenocoumarol which is cleared 3-4 times faster and is primarily metabolized by CYP2C9 and hydroxylated at the 6-, 7-, and 8 position [132,123-124,126-127,138].

R-acenocoumarol is absorbed from the gastrointestinal tract with almost complete oral bioavailability, unlike S-acenocoumarol which undergoes of extensive first pass metabolism. Within a few hours, peak plasma concentrations are reached, the correlation between plasma concentrations of acenocoumarol and apparent prothombin levels cannot be established due to the variation of plasma drug concentrations between patients, plasma drug concentrations are generally higher among patients of 70 years and above when compared to the younger patients. Elimination of both CAN isomers depends entirely on hepatic biotransformation, 29% of the drug is excreted in the form of faeces and 60% in urine [122,125,128].

Compound	Primary dose (mg)	Subsequent dose (mg)	action
Warfarin	30-40	10	Moderately rapid transit, easy to control
Acenocoumarol	15-25	2-10	Moderately rapid effect, rapid exit
Phenprocoumon	20-30	0.75-6	Slow effect, slow recovery

Table 3: structure and dose response relationship of some OAC.

12. Conclusion:

In this chapter, we have studied a brief overview of thromboembolic diseases and the role of coumarin with a general anticoagulant effect, and we have specified that acenocomarol was the main focus of this study, the active ingredient of the drug Sintrom, which is usually prescribed to patients with heart failure, stroke or other embolisms. We have also discussed previous studies on this subject, as a chemical compound and its synthesis methods, as well as some of its spectral properties, and provide a little overview of its chemical and physical properties. Finally, we also mentioned the importance of this class of drugs on the current market and the reasons for its medical uses, despite the discovery of new anticoagulants with different properties and advantages.

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