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DESIGN, SYNTHESIS AND ANTICOAGULANT ACTIVITY OF SOME NOVEL COUMARINS

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ABSTRACT

Objectives: Synthesis and *in vitro* anti-coagulant activity of some novel coumarin derivatives. The need for anti-coagulant drugs is because the Stroke, heart attack, and pulmonary embolism are extremely common and often fatal conditions. **Methods:** Coumarin derivatives were prepared by the condensation of 4-hydroxy coumarin or 7-hydroxy 4- methyl coumarin with various acylated aromatic amines in the presence of dry acetone as solvent. The acylated aromatic amines are prepared from various aromatic amines by treating with chloro acetyl chloride and acetic acid. **Results:** Ten coumarin derivatives (CPD-1- CPD-10) are synthesized characterized by NMR and mass spectral data and tested for in vitro anticoagulant activity by taking warfarin as standard. **Conclusion:** Coumarin derivatives are showing the moderate anticoagulant activity. Further can be tested for other biological activities.

KEYWORDS

Coumarin, anticoagulant, 7-hydroxy 4- methyl coumarin, 4-hydroxy coumarin, Acetone and Acylated aromatic amines.

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INTRODUCTION

Coumarin (2H-chromen-2-one) is a fragrant chemical compound in the benzopyroneical class, found in many plants, notably in high concentration in the tonka bean (Dipteryx odorata), vanilla grass (Anthoxanthumodoratum), sweet woodruff (Galiumodoratum), mullein (Verbascum s pp.), sweet grass (Hierochloe odorata), cassia cinnamon (Cinnamomum aromaticum) and sweet clover (Fabaceae spp.)[1]. The name comes from a

French word, coumarou, for the tonka bean. It has a sweet odor, readily recognized as the scent of newly-mown hay, and has been used in perfumes since 1882[1]. Sweet woodruff, sweet grass and sweet clover in particular are named for their sweet smell, which is due to their high content of this substance. It has been used as an aroma enhancer in pipe tobaccos and certain alcoholic drinks, although in general it is banned as a flavorant food additive, due to concerns about hepatotoxicity coumarin causes in animal models[1]. When it occurs in high concentrations in forage plants, coumarin is a somewhat bitter-tasting appetite suppressant, and is presumed to be produced by plants as a defense chemical to discourage predation[1]. Although coumarin itself has no anticoagulant properties, it is transformed into the natural anticoagulant dicoumarol by a number of species of fungi. This occurs as the result of the production of 4-hydroxycoumarin, then further (in the presence of naturally occurring formaldehyde) into the actual anticoagulant dicoumarol, a fermentation product and mycotoxin[1]. This substance was responsible for the bleeding disease known historically as "sweet clover disease" in cattle eating moldy sweet clover silage[1]. Coumarin is used in the pharmaceutical industry as a precursor molecule in the synthesis of a number of synthetic anticoagulant pharmaceuticals similar to dicoumarol, the notable ones being warfarin (trade named "Coumadin," not to be confused with coumarin) and some even more potent rodenticides that work by the same anticoagulant mechanism[1].

Coumarin has clinical medical value by itself, as an edema modifier. Coumarin and other benzopyrones, such as 5, 6 benzopyrone, 1, 2 benzopyrone, diosmin, and others, are known to stimulate macrophages to degrade extracellular albumen, allowing faster resorption of edematous fluids. Other biological activities that may lead to other medical uses have been suggested, with varying degrees of evidence [1].Coumarin is also used as a gain medium in some dye lasers, and as a sensitizer in older photovoltaic technologies. The biosynthesis of Coumarin in plants is via hydroxylation, glycolysis, and cyclization of cinnamic acid. Coumarin can be prepared in a laboratory in a Perkin reaction between salicylaldehyde and acetic anhydride[1]. The Pechmann condensation provides another synthesis of coumarin and its derivatives[1].

The benzo-2-pyrone nucleus of the simple coumarins derives from the phenylacrylic skeleton of cinnamic acids[1].

Pharmacological and biochemical actions of simple coumarins

More than 300 coumarins have been identified from natural sources, especially green plants. The pharmacological and biochemical properties and therapeutic applications of simple coumarins depend upon the pattern of substitution. More complex related compounds based on the coumarin nucleus include the dicoumarol/warfarin anticoagulants, aflatoxins and the psoralens (photosensitizing agents)[2]. Coumarin itself (1, 2-benzopyrone) has long-established efficacy in slow-onset long-term reduction of lymphoedema in man, as confirmed in recent double-blind trials against elephantiasis and post mastectomy swelling of the arm. The mechanism of action is uncertain, but may involve macrophage-induced proteolysis of oedema protein. However, coumarin has low absolute bioavailability in man (< 5%), due to extensive first-pass hepatic conversion to 7-hydroxycoumarin followed by glucuronidation. It may, therefore, be a prodrug[2]. Scoparone (6, 7-dimethoxycoumarin) has been purified from the hypolipidaemic Chinese herb Artemisia scoparia and shown to reduce the proliferative responses of human peripheral mononuclear cells, to relax smooth muscle, to reduce total cholesterol and triglycerides and to retard the characteristic pathomorphological changes in hypercholesterolaemic diabetic rabbits. Various properties of scoparone were suggested to account for these findings, including ability to scavenge reactive oxygen species, inhibition of tyrosine kinases and potentiation of prostaglandin generation[2]. Osthole (7-methoxy-8-[3-methylpent-2-enyl] coumarin) from Angelica pubescens, used also in Chinese medicine, causes hypotension *in vivo,* and inhibits platelet aggregation and smooth muscle contraction *in vitro*[2]. It may interfere with

calcium influx and with cyclic nucleotide phosphodiesterases. Cloricromene, a synthetic coumarin derivative, also possesses antithrombotic antiplatelet actions, inhibits PMN neutrophil function and causes vasodilatation. Some of these properties of cloricromene have been ascribed to inhibition of arachidonate release from membrane phospholipids[2].

Chemistry of coumarins

Chemistry

Coumarin or 2H-chromen-2one has also been named like coumadin, benzo-1, 3-diazine or 5, 6,-benzopyrimidine. It was first prepared by Gabriel in 1903.

Coumarin and its derivatives are all considered phenylpropanoids. Some naturally occurring Coumarin derivatives include umbelliferone (7-hydroxycoumarin), aesculetin (6, 7dihydroxycoumarin), herniarin (7-methoxycoumarin), psoralen and imperatorin. 4Phenylcoumarin is the backbone of the neoflavones, a type of neoflavonoids.

Coumarin nucleus in ball and stick model

Synthesis

Resorcinol (1.1 g) was dissolved completely in ethyl acetoacetate (1.35 g) in a 50 ml dry round bottom flask. 40 ml of con sulphuric acid was added to this homogeneous mixture and mixed thoroughly using a glass rod. It was placed on a hot water bath and heated gently for 3-4 h. After completion of the reaction, mixture was cooled to room temperature and 7-hydroxy-4-methylcoumarin was extracted[34,35.]

Biological activity

Coumarins are a group of important natural compounds, and have been found to have multi-biological activities such as anticoagulant, anti-HIV, anti-tumor, anti-hypertension, anti-arrhythmia, anti-osteoporosis, assuaging pain, preventing asthma and antisepsis. Therefore, further investigation should emphasize on improving techniques for their development towards efficacy, safety and potency.

Since, coumarins are known to possess anticoagulant, antiproliferative, antimicrobial, spasmolytic, antitumor, antioxidant activities attempt will be made to design, synthesize, purify, analyze and evaluate for their possible anticoagulant activity. In view these findings we propose to design synthesize new coumarin derivatives and evaluate for their possible anticoagulant activity.

MATERIAL AND METHODS

Chemicals and Reagents

7-hydroxy-4-methyl coumarin was prepared in JSS college of Pharmacy laboratory and reagents solvents used are purchased from MERCK, Mumbai. The reagents, chemicals and solvents used for the present work of laboratory and analytical grade and purified by standard procedures reported in Vogel's text book of practical organic chemistry. The melting point of the synthesized compounds was determined in open capillary method, expressed in $^{\circ}C$. A periodic monitoring of the reaction was made through TLC (Prepared with silica gel G) with the solvent systems pet ether: ethyl acetate: chloroform in the ratio of 5.5: 0.5: 4 and pet ether: ethyl acetate in the ratio 6:4.

IR spectra were recorded on Shimadzu FT-IR 8400-S spectrophotometer (JSS College of Pharmacy) by KBr pellet technique and are expressed in cm^{-1}. $^{1}HNMR$ and $^{13}CNMR$ spectra were recorded on Bruker 400 MHz FT-NMR spectrophotometer (IIT MADRAS) using DMSO (d_6) as the solvent and TMS as internal standard (δ ppm). Mass spectra were obtained by both GC-MS JEOL GC-MATE II, Under EI ionization at 70ev (IIT MADRAS).Rats for in vivo study are taken from animal house of JSS college of Pharmacy, Mysore with animal ethical committee.

Permission

In the present study we prepared some novel coumarins by adopting suitable synthetic schemes. We have optimized the synthetic schemes and the synthesized compounds were purified and subjected for physical and spectral analysis.

Experimented in the year 2012 at JSS College of Pharmacy, Mysore.

Chemical synthesis

Preparation of 7-hydroxy-4-methyl coumarin

20ml of con H_2SO_4 was placed in an iodine flask and maintained the temperature to 0-5° C. Mixture of equimolar quantity of ethyl

acetoacetate (3.35ml) and resorcinol (2.8 g) was transferred to the iodine flask containing sulphuric acid and stirred until a clear solution is obtained under ice cold conditions for about 2 h. The reaction was monitored through TLC. After completion of the reaction the reaction mixture was poured in to the beaker containing crushed ice and stirred well to form the crude precipitate of 7-hydroxy-4-methyl-coumarin. Further, filtered and recrystallized from ethanol.

Preparation of Acylated aromatic amines

Aromatic amine (0.1mole) and 10 ml of acetic acid was taken in a iodine flask kept in a ice-cold conditions. 8 ml of chloroactylchloride in 10 ml of actic acid was added from dropping funnel with continuous stirring for 2h. The reaction was monitored through the TLC. Product formation is confirmed by TLC and MP.

Preparation of coumarin derivatives (Compounds CPD-1-CPD-10)

Acylated amine (0.02mole) and coumarin (4-hydroxy coumarin or 7-hydroxy-4-methyl coumarin, 0.04 mole) were taken in a round bottom flask along with 15 ml of dry acetone as solvent .0.04 moles of potassium carbonate was added and stirred for 48-60 h at 50°C until the product formation. The reaction was monitored through TLC. Then filtered, the filtrate was evaporated, the solid obtained was washed for two times with 5% sodium hydroxide solution and with water to get the crude product. Then recrystallised with ethanol to get pure compound.

The final compounds were characterized by TLC, MP, IR, ^{1}HNMR, ^{13}CNMR and Mass spectral analysis as shown in the Results section.

Anticoagulant activity

Anticoagulants have been widely used both clinically and *in vitro* medical treatments. In clinical practice, they are the drugs of choice for the prevention and treatment of thromboembolic disorders, and prophylaxis of thrombotic events both pre- and post-surgery. Warfarin is an available anticoagulant in the market which acts by inhibiting vitamin K-dependent factors. Since, our synthesised compounds are also coumarin derivatives we have screened for their possible anticoagulant activity using albino rats by following the procedure described below after grouping the animals.

Grouping

Rats of both the sex of 12 numbers were used, weighed and housed in an animal house. The animals were maintained on a commercial standard pellet diet and water under standard conditions. Rats were randomized into six groups each consisting of two rats, one in each group for the dose of 1mg/kg body weight and 0.5 mg/kg body weight, respectively. The experiment was performed in two batches for all the ten synthesized compounds using the standard drug as warfarin. Each rat in a group was identified by permanent marking with Picric acid as,

1. Head
2. Head and Body

PROCEDURES

Preparation of test compounds and standard drug solutions and dosing

All the synthesized compounds (CPD-1-CPD-10) and standard drug, Warfarin, were dissolved in water and suspended with the help of a CMC to prepare the standard stock solution containing 0.1mg/ml. This stock solution was used to inject each rat of the respective group by oral route. Out of the two rats in each group, each one was administered at the dose of 0.5mg/kg body weight and 1.0 mg/kg body weight[34].

Determination of clotting time/anticoagulant activity

The clotting time was determined by capillary tube method, it is a time taken to form a visible clot in the form of fibrin which is measured by breaking the capillary tube at regular intervals. Blood was collected from the retro orbital plexuses under light ether anesthesia. The clotting time of the blood before injecting the sample was considered as a normal. Further after every 6 hours the clotting time of the blood was determined till the 48 h[32].

RESULTS AND DISCUSSION

The coumarin derivatives were prepared according to the synthetic scheme outlined in the materials and

methods and the physical data of the compounds synthesized are provided.

Anticoagulant activity

The *in vivo* anti-coagulant activity of the synthesized compounds were determined by capillary tube method the results of this evaluation are summarized in Ta.

DISCUSSION

Designing some novel coumarins for their possible anticoagulant activity

Considering the side effects such as blood in the urine, bleeding gums, unusual bleeding, lethargy, weakness etc associated with Warfarin, an existing drug in the market as oral anticoagulant gives us the opportunity to develop some newer coumarins for their anticoagulant properties. In this context, considering the structure of Warfarin some novel coumarin derivatives were designed considering the synthetic feasibility in our laboratory. As the warfarin structure contains an hydrophobic substitution at the third position of its coumarin ring system. Here, we have made an attempt to derivatise the phenolic hydroxyl group of the coumarin nucleus at fourth and seventh positions. In this way, we chose different aromatic amines and connected with two carbon linker to connect it to the phenolic hydroxyl group of coumarin nucleus. We have designed two series of compounds, one with substitution on the phenolic hydroxyl group of 4-hydroxy coumarin and another series over the phenolic hydroxyl group oh 7-hydroxy-4-methyl coumarin.

Chemistry

As part of the synthesis, 7-hydroxy4-methyl coumarin were synthesized by reacting resorcinol with ethylacetoacetate in acidic medium in good to moderate yields as shown in the synthetic Scheme. The acylated aromatic amines were synthesized by reacting chloroacetylchloride with different aromatic amines. Then coumarin derivatives (CPD-1-CPD-10) were prepared by reacting 7-hydroxy4-methylcoumarin (or) 4-hydroxycoumarin with different acylated aromatic amines. The compounds were obtained in good yields (60-85%) and the purity of the compounds was established by single spot in TLC. The compounds synthesized were characterized by IR, NMR and Mass spectral data.

Synthesis of coumarin derivatives (CPD-1-CPD-5): The fusion of 0.02mole of different acylated aromatic amines and 0.04 mole of 4-hydroxy coumarinn in the presence of 0.02 mole of potassium carbonate afforded the coumarin derivatives (CPD-1-CPD-5) with 60% of isolated yield

IR spectrum of coumarin derivatives(4-hydroxy coumarin derivatives) (CPD-1-CPD-5) exhibited a broad absorption at 3300 cm-1 to 3500 cm-1 which indicates the presence of the amine group. The aromatic C-H stretching was observed at 3000 cm-1to3100 cm-1. The aliphatic C-H stretching was observed at 2900 cm-1 to 2999 cm-1.The compound 2-(2-oxo-2H-chromen-4-yloxy)-N-(4-chlorophenyl) acetamide exhibited a band at 709 cm-1 is attributed to the chloro group in the nucleus.

IR spectrum of coumarin derivatives(7-hydroxy 4-methyl coumarin derivatives) (CPD-6-CPD-10) exhibited a broad absorption at 3300 cm-1 to 3500 cm-1 which indicates the presence of the amine group. The aromatic C-H-stretching was observed at 3000 cm-1to3100 cm-1. The aliphatic C-H stretching was observed at 2900 cm-1 to 2999 cm-1. The compound 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N-(4-fluorophenyl)acetamide (CPD-8) exhibit absorption at 775 cm-1 indicates that it contains halogen (F) atom in nucleus. The compound 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N-(4-chlorophenyl) acetamide (CPD-10) is showing absorbance at 776 cm-1 indicates that it contain chlorine group in nucleus.

The 1H-NMR spectrum of 2-2-(2-oxo-2H-chromen-4-yloxy)-N-m-tolylacetamide (CPD-1) resonated at singlet at 10.18 ppm attributed to NH group one proton gives singlet peak. The aromatic protons resonated as multiplet between 6.9-9.8 ppm. The resonance peaks at 5.89, 5.02, 2.3 ppm indiacates =CH, CH2, CH3 respectively. The 13C-NMR spectrum of 2-2-(2-oxo-2H-chromen-4-yloxy)-N-m-tolylacetamide (CPD-1) resonated at 21.09 indicates CH3 carbon atom and peak at 67 ppm indicates CH2 carbon atom. Resonance peaks between 115

ppm to 161 ppm indicates 13 aromatic carbon atoms and peaks at 164.36 ppm, 164.59 ppm indicates two C=O group carbon atoms. The GC-MS spectrum of 2-2-(2-oxo-2H-chromen-4-yloxy)-N-m-tolylacetamide (CPD-1) showed molecular ion peak at m/e 309.32.

Structural Cleavage of 2-2-(2-oxo-2H-chromen-4-yloxy)-N-m-tolylacetamide (CPD-1) for Mass spectra

The 1H-NMR spectrum of 2-(2-oxo-2H-chromen-4-yloxy)-N-p-tolylacetamide (CPD-2) resonated at singlet at 9.63 ppm attributed to NH group one proton gives singlet peak. The aromatic protons resonated as multiplet between 6.4-7.5 ppm. The resonance peaks at 4.72 ppm, 3.98? ppm, 2.25ppm indicates =CH,CH2,CH3 respectively. The 13C-NMR spectrum of 2-(2-oxo-2H-chromen-4-yloxy)-N-p-tolylacetamide (CPD-2) resonated at 20.36 indicates CH3 carbon atom and peak at 58.57 ppm indicates CH2 carbon atom. Resonance peaks between 114 ppm to 135 ppm indicates 13 aromatic carbon atoms and peaks at 167.72 ppm indicates two C=O group carbon atoms. The GC-MS spectrum of 2-(2-oxo-2H-chromen-4-yloxy)-N-p-tolylacetamide (CPD-2) showed molecular ion peak at m/e 309.

Structural Cleavage of 2-(2-oxo-2H-chromen-4-yloxy)-N-p- tolylacetamide (CPD-2) for Mass spectra

The GC-MS spectrum of Mass spectrum of 2-(2-oxo-2H-chromen-4-yloxy)-N-(4- chlorophenyl) acetamide (CPD-3) showed molecular ion peak at m/e 329.

Structural Cleavage of2 - (2-oxo-2H-chromen-4-yloxy)-N-(4- chlorophenyl) acetamide (CPD-3) for **Mass spectra**

The 13C-NMR spectrum of 2-(2-(2-oxo-2H-chromen-4-yloxy) acetamido) benzoate (CPD-5) resonated at 18.36 indicates CH3 carbon atom and peak at 58.57 ppm indicates CH2 carbon atom. Resonance peaks between 116 ppm to 135 ppm indicates 13 aromatic carbon atoms and peaks at 167.72 ppm indicates two C=O group carbon atoms. The GC-MS spectrum of Mass spectrum of 2-(2-(2-oxo-2H-chromen-4-yloxy) acetamido) benzoate (CPD-5) showed molecular ion peak at m/e 353.

Structural Cleavage of 2-(2-(2-oxo-2H-chromen-4-yloxy) acetamido) benzoate (CPD-5) for Mass spectra.

The 1H-NMR spectrum of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N-m-tolylacetamide (CPD-6) resonated at singlet at 10.10 ppm attributed to NH group one proton gives singlet peak. The aromatic protons resonated as multiplet between 6.88-7.72 ppm. The resonance peaks at 6.21 ppm, 4.83 ppm, 2.49 ppm, 2.25 ppm indicates =CH, CH2, CH3, CH3 respectively. The 13C-NMR spectrum of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N-m-tolylacetamide (CPD-6) resonated at 18.08 indicates CH3 carbon atom and peak at 21.11 ppm indicates CH2 carbon atom. Resonance peaks between 111.40 ppm to 160 ppm indicates 13 aromatic carbon atoms and peaks at 160.83 ppm,165.72 ppm indicates two C=O group carbon atoms. The GC-MS spectrum of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N-m-tolylacetamide (CPD-6) showed molecular ion peak at m/e 323.

Anti-coagulant activity

Anticoagulants have been widely used both clinically and in vitro medical treatments. In clinical practice, they are the drugs of choice for the prevention and treatment of thromboembolic disorders, and prophylaxis of thrombotic events both pre- and post-surgery. Warfarin is an available anticoagulant in the market which acts by inhibiting vitamin K-dependent factors. Since, our synthesised compounds are also coumarin derivatives we have screened for their possible anticoagulant activity using albino rats by following the procedure described below after grouping the animals. All the synthesized compounds (CPD-1to CPD-10) were screened for their possible anti-coagulant activity using clotting time measurement on albino rats after injecting the compounds at the two dose levels of 0.5mg/kg body weight and 1.0mg/kg body weight, respectively. *In vivo* anticoagulent activity of the synthesized compounds was determined by the capillary method using warfarin as standard anticoagulent. Surprisingly, none of the synthesized coumarins showed significant anticoagulant

activity. However, compounds CPD-1, CPD-2, CPD-5 and CPD-10 showed a moderate anticoagulant activity. Whereas rest of the compounds exhibited no anticoagulant activity.

Table No.1: List of aryl amines used for the preparation of acylated amines

Sym	Aryl amine	R
CPD-1	m-toluidine	CH_3
CPD-2	p-toluidine	CH_3
CPD-3	4-chloroaniline	Cl
CPD-4	o-toluidine	CH_3
CPD-5	methyl 2-aminobenzoate	CH_3COO
CPD-6	m-toluidine	CH_3
CPD-7	p-toluidine	CH_3
CPD-8	4-fluoroaniline	F
CPD-9	Aniline	-
CPD-10	4-chloroaniline	Cl

Table No.2: List of Synthesized Compounds

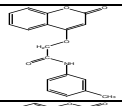
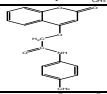
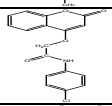
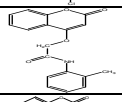
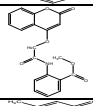
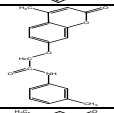
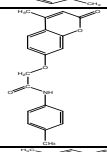
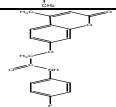
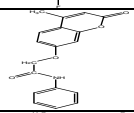
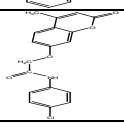
Sym	Structure	Chemical name
CPD-1		2-(2-oxo-2H-chromen-4-yloxy)-N-m-tolylacetamide
CPD-2		2-(2-oxo-2H-chromen-4-yloxy)-N-p-tolylacetamide
CPD-3		2-(2-oxo-2H-chromen-4-yloxy)-N-(4-chlorophenyl) acetamide
CPD-4		2-(2-oxo-2H-chromen-4-yloxy)-N-o-tolylacetamide
CPD-5		Methyl 2-(2-(2-oxo-2H-chromen-4-yloxy) acetamido) benzoate
CPD-6		2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N-m-tolylacetamide
CPD-7		2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N-p-tolylacetamide
CPD-8		2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N-(4-fluorophenyl)acetamide
CPD-9		2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N-phenylacetamide
CPD-10		2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N-(4-chlorophenyl)acetamide

Table No.3: Physical data of the Synthesized compounds CPD-1 to CPD-5 from 4-Hydroxy Coumarin

Sym	Molecular formula	M.W.	m.p. (°C)	R_f value	% Yield
CPD-1	$C_{18}H_{15}NO_4$	309	275	0.61*	82
CPD-2	$C_{18}H_{15}NO_4$	309	289	0.87*	75
CPD-3	$C_{17}H_{12}ClNO_4$	330	268	0.82**	85
CPD-4	$C_{18}H_{15}NO_4$	309	293	0.62**	80
CPD-5	$C_{19}H_{15}NO_6$	353	278	0.81**	72

*Mobile phase = toluene: ethyl acetate (6: 4)

**Mobile phase = ethyl acetate: toluene: chloroform (0.5:5.5:4)

Table No.4: Physical data of the compounds 6 to 10 from 7-Hydroxy-4-methyl Coumarin

Sym	Molecular formula	M.W.	m.p. (°C)	R_f value	% Yield
CPD-6	$C_{19}H_{17}NO_4$	323	296	0.71*	82
CPD-7	$C_{19}H_{17}NO_4$	323	291	0.53**	75
CPD-8	$C_{18}H_{14}FNO_4$	327	295	0.23**	70
CPD-9	$C_{18}H_{15}NO_4$	309	290	0.49*	55
CPD-10	$C_{18}H_{15}ClNO_4$	344	265	0.67*	70

*Mobile phase = toluene: ethyl acetate (6: 4)

**Mobile phase = ethyl acetate: toluene: chloroform (0.5:5.5:4)

Table No.5: IR spectral data of the synthesized compounds

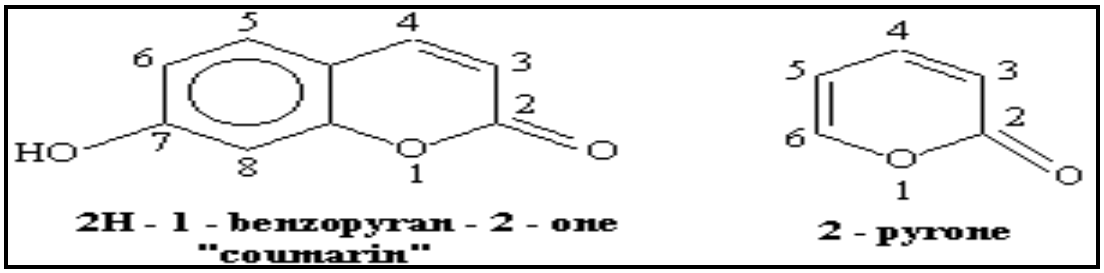
Sym	CHEMICAL NAME	IR data in cm^{-1}
CPD-1	2-(2-oxo-2H-chromen-4-yloxy)-N-m-tolylacetamide	3390(N-H), 3080(ArC-H), 2950(AliCH), 1691(C=O), 1678(C=O), 1602(C=C), 1263 (C-O)
CPD-2	2-(2-oxo-2H-chromen-4-yloxy)-N-p-tolylacetamide	3375(N-H), 3032(ArC-H), 2922(Ali-CH), 1672(C=O), 1651(C=O), 1647(C=C)1112 (C-O)
CPD-3	2-(2-oxo-2H-chromen-4-yloxy)-N-(4-chlorophenyl) acetamide	3322(N-H), 3064(ArC-H), 2922(Ali-CH), 1695(C=O), 1681(C=O), 164720(C=C)1192(C-O), 762 (C-Cl)
CPD-4	2-(2-oxo-2H-chromen-4-yloxy)-N-o-tolylacetamide	3301(N-H), 3090(ArC-H), 2962(Ali-CH), 1693(C=O), 1627(C=O), 1608(C=C), 1188 (C-O)
CPD-5	Methyl 2-(2-(2-oxo-2H-chromen-4-yloxy) acetamido) benzoate	3369(N-H), 3070(ArC-H), 2980(Ali-CH), 1678(C=O), 1624(C=O), 1606(C=C), 1188 (C-O), 1122(C-O)
CPD-6	2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N-m-tolylacetamide	3363(N-H), 3095(ArC-H), 2985(-CH_3), 2914(Ali-CH), 1687(C=O), 1672(C=O), 1620(C=C), 1155 (C-O)
CPD-7	2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N-p-tolylacetamide	3300(N-H), 3097(ArC-H), 2970(-CH_3) 2920(Ali-CH), 1121 (C-O)
CPD-8	2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N-(4-fluorophenyl) acetamide	3423(N-H), 3059(ArC-H), 2899(-CH_3), 2914(Ali-CH), 1697(C=O), 1676(C=O), 1654(C=C), 1161 (C-O),795(-F)
CPD-9	2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N-phenylacetamide	3321(N-H), 3064(ArC-H), 2800(-CH_3), 2900(Ali-CH), 1696(C=O), 1681(C=O), 1620(C=C), 1141 (C-O)
CPD-10	2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-N-(4-chlorophenyl) acetamide	3423(N-H), 3051(ArC-H), 2890(-CH_3), 2920(Ali-CH), 1672(C=O),1670(C=O), 1635(C=C), 1154 (C-O), 776(-Cl)

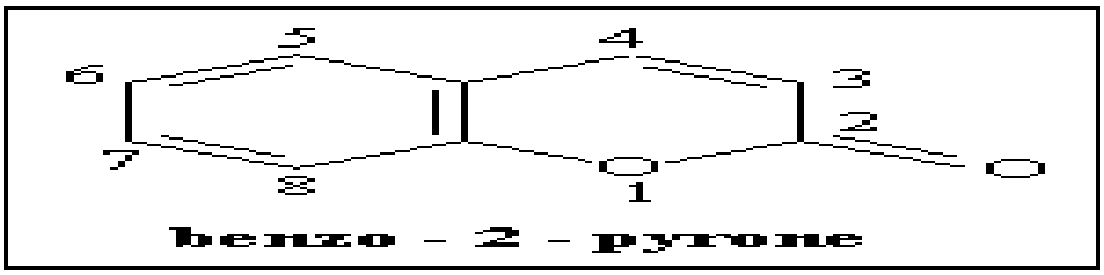
Table No.6: ^{1}H-NMR, C^{13}-NMR, Mass Spectral data of Synthesized Compounds

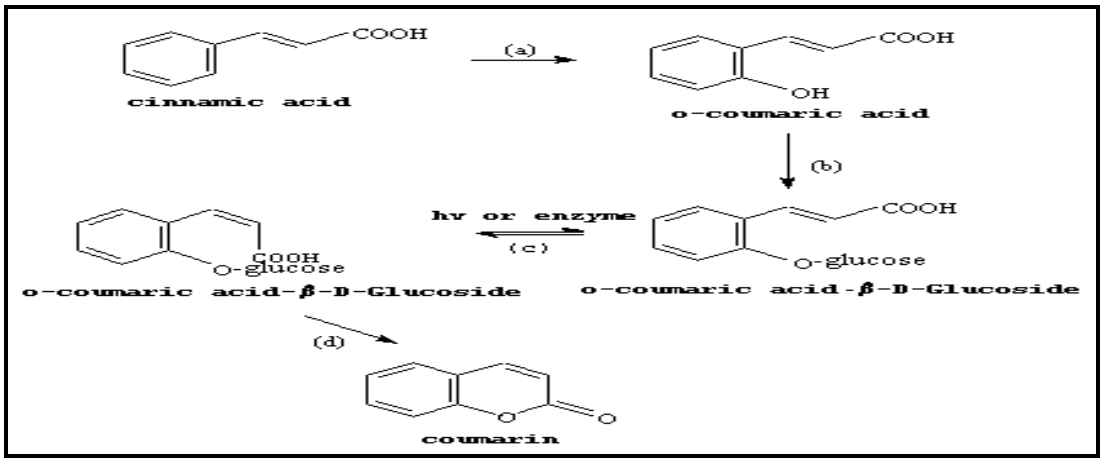
Sym	^{1}H-NMR Spectral data		C^{13}-NMR Spectral data		Mass Spectral data
	Chemical shift (δ ppm)	Protons	Peaks at (δ ppm)	Carbons	
CPD-1	10.18 6.9-7.9 5.89 5.02 2.30	(s, 1H, NH) (m, 8H, Ar-H) (s, 1H, =CH) (s, 3H, CH_2) (s, 3H, CH_3)	21.09 67.66 91.18 115-161 164.36 164.59	(CH_3) (CH_2) (=CH_2) (13 Ar-C) (C=O) (C=O)	61.73 (base peak) 309 (M^+, 80%) 311 (M+2, 25%)
CPD-2	9.63 7.54 7.15 7.05-6.4 4.73 3.9 2.25	(S, 1H, NH) (d, 2H, NH) (d, 2H, Ar-H) (m, 4H, Ar-H) (s, 1H, =CH) (s, 2H, CH_2) (s, 3H, CH_3)	20.36 58.57 71.68 114-135 167.72 167.72	(CH_3) (CH_2) (=CH_2) (13 Ar-C) (C=O) (C=O)	61.43 (base peak) 309 (M^+, 80%) 311 (M+2, 25%)
CPD-3	___	___	___	___	62 (base peak) 329 (M^+, 80%) 331 (M+2, 25%)
CPD-5	___	___	19 57.35 90.08 116-135 161.72 167.83	(CH_3) (CH_2) (=CH_2) (13 Ar-C) (C=O) (C=O)	57 (base peak) 353 (M^+, 80%) 355 (M+2, 25%)
CPD-6	10.10 7.72-6.88 6.21 4.83 2.49 2.26	(s, 1H, NH), (m, 7H, Ar-H), (s, 1H, =CH), (s, 2H, CH_2) (s, 3H, CH_3) (s, 3H, CH_3)	18.08 21.11 67.27 101.66 111.40-160 160.72 167.83	(CH_3) (CH_3) (CH_2) (=CH) (13Ar-C) (C=O) (C=O)	58 (base peak) 323 (M^+, 80%) 325 (M+2, 25%)

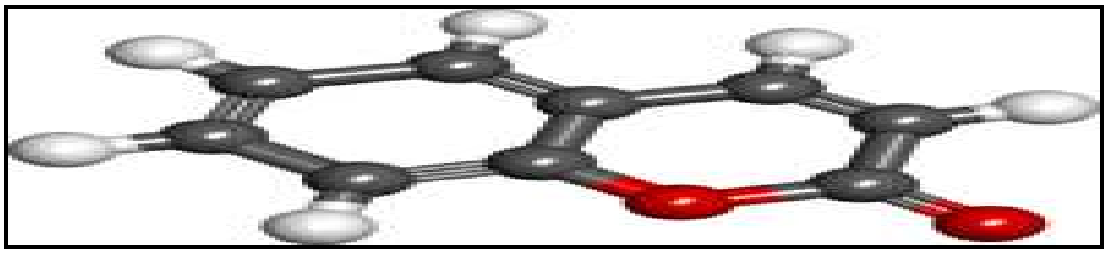
Table No.7: *In-vivo* anti-coagulant activity data (capillary tube method of anti-coagulant activity)

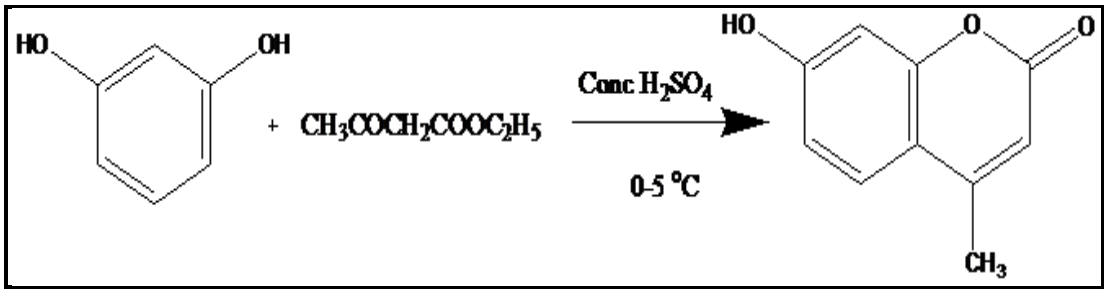
Groups of Rats		CLOTTING TIME (IN SEC)								
	Duration	0 h	6 H	12 h	18 h	24 h	30 h	36 h	42 h	48 h
Group-1	Standard drug (Warfarin 1mg/kg)	30	128	186	254	310	360	385	349	320
Group-2	CPD-1 (0.5mg/kg)	28	55	60	75	80	105	110	97	80
	CPD-1 (1.0mg/kg)	30	80	92	120	158	178	180	130	115
Group-3	CPD-2 (0.5mg/kg)	24	90	98	100	110	115	120	123	100
	CPD-2 (1.0mg/kg)	31	98	100	125	130	140	145	150	130
Group-4	CPD-3 (0.5mg/kg)	25	28	31	33	39	41	42	36	33
	CPD-3 (1.0mg/kg)	27	31	34	36	41	45	42	38	31
Group-5	CPD-4 (0.5mg/kg)	29	32	33	34	35	31	29	28	27
	CPD-4 (1.0mg/kg)	31	34	36	40	43	44	39	38	36
Group-6	CPD-5 (0.5mg/kg)	24	100	120	120	130	135	140	135	120
	CPD-5 (1.0mg/kg)	28	115	156	160	170	180	185	180	140
Group-1	CPD-6 (0.5mg/kg)	25	27	31	35	37	40	41	35	34
	CPD-6 (1.0mg/kg)	31	35	35	39	41	43	42	39	38
Group-2	CPD-7 (0.5mg/kg)	29	31	33	35	40	43	39	37	36
	CPD-7 (1.0mg/kg)	31	33	35	38	41	45	42	41	38
Group-3	CPD-8 (0.5mg/kg)	28	30	33	36	37	42	45	40	39
	CPD-8 (1.0mg/kg)	33	34	38	41	44	46	41	38	36
Group-4	CPD-9 (0.5mg/kg)	26	29	34	37	39	40	41	38	38
	CPD-9 (1.0mg/kg)	30	34	38	39	43	44	42	38	36
Group-5	CPD-10 (0.5mg/kg)	26	70	80	86	80	90	98	85	80
	CPD-10 (1.0mg/kg)	30	80	81	86	98	100	110	120	90

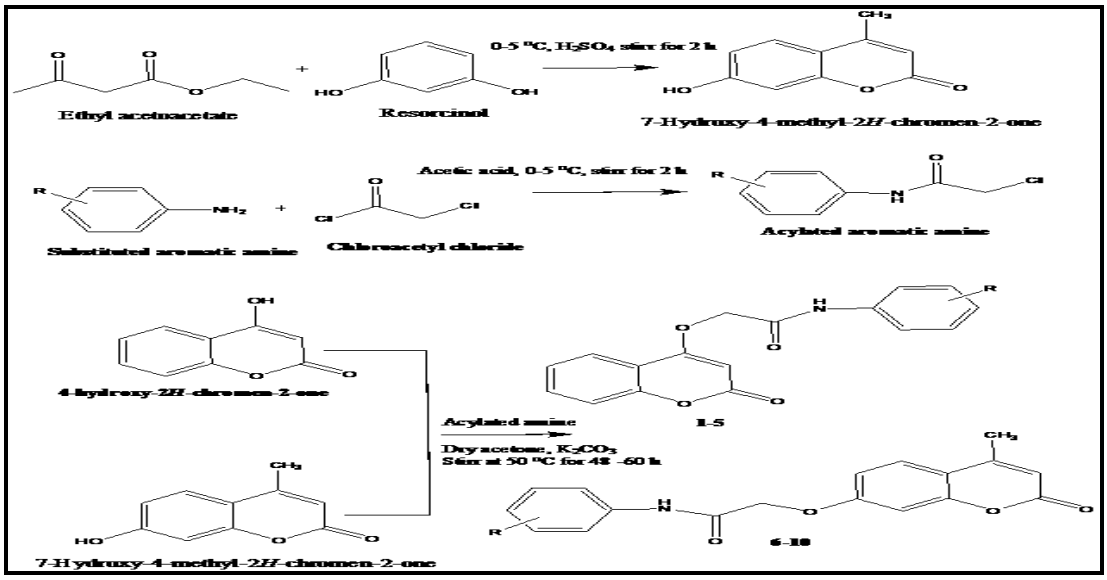

2H - 1 - benzopyran - 2 - one
"coumarin"
2 - pyrone


benzo - 2 - pyrone

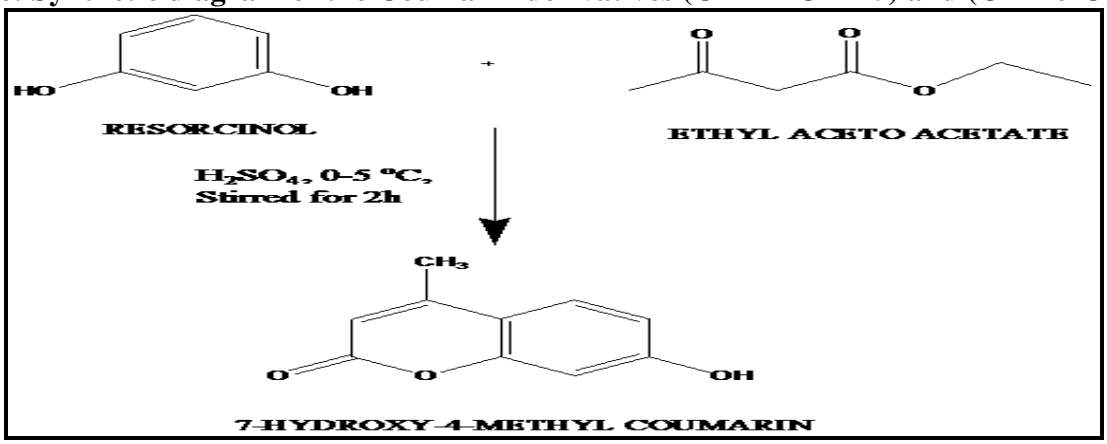

cinnamic acid
(a)
o-coumaric acid
(b)
hv or enzyme
(c)
o-coumaric acid-β-D-Glucoside
o-coumaric acid-β-D-Glucoside
(d)
coumarin



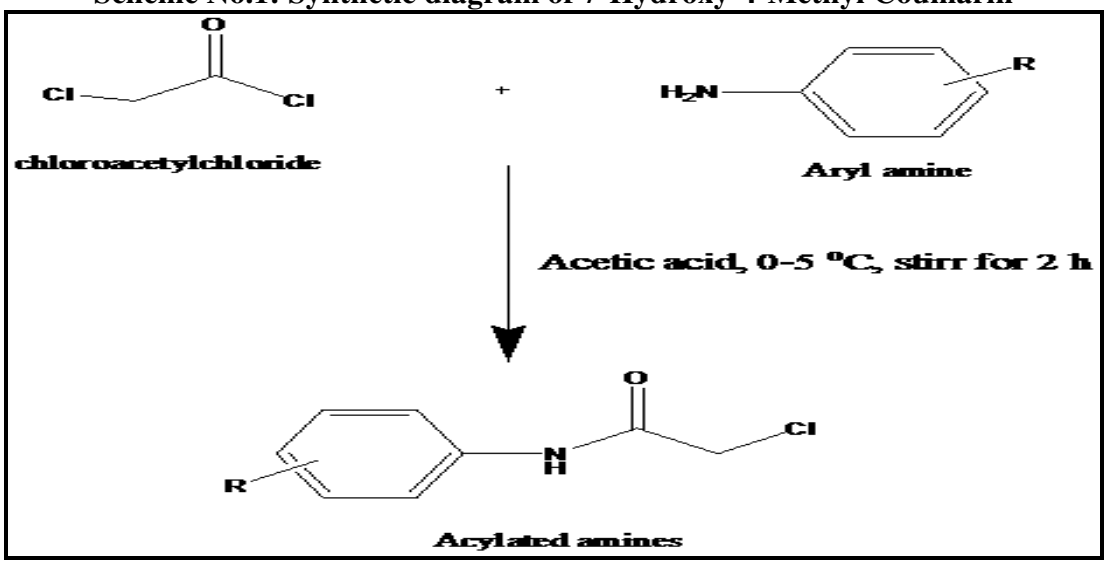

Conc H2SO4
0-5 °C



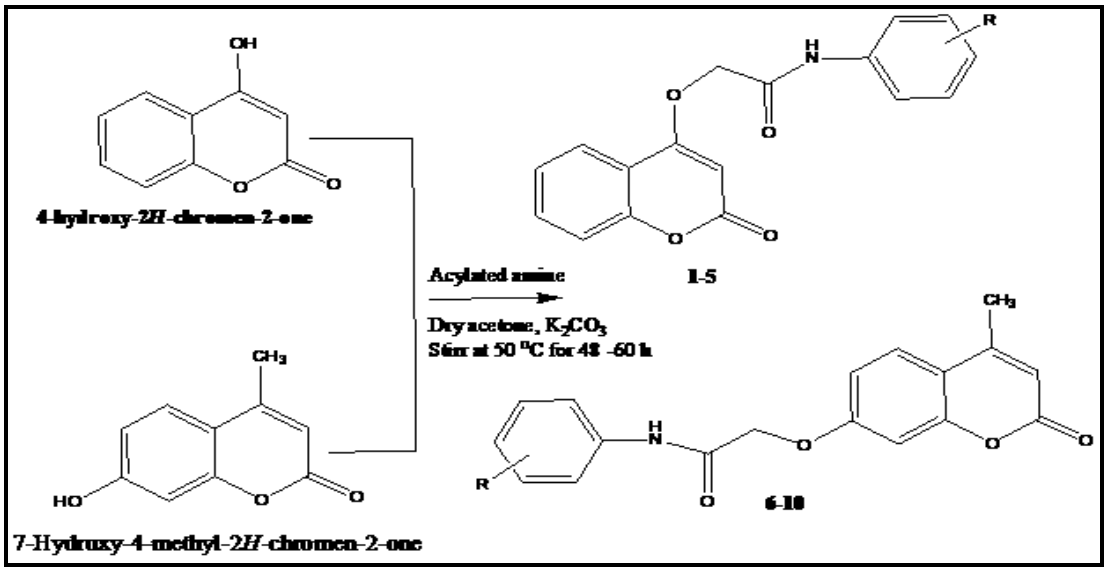
Scheme: Synthetic diagram of the Coumarin derivatives (CPD-1-CPD-5) and (CPD-6-CPD-10)



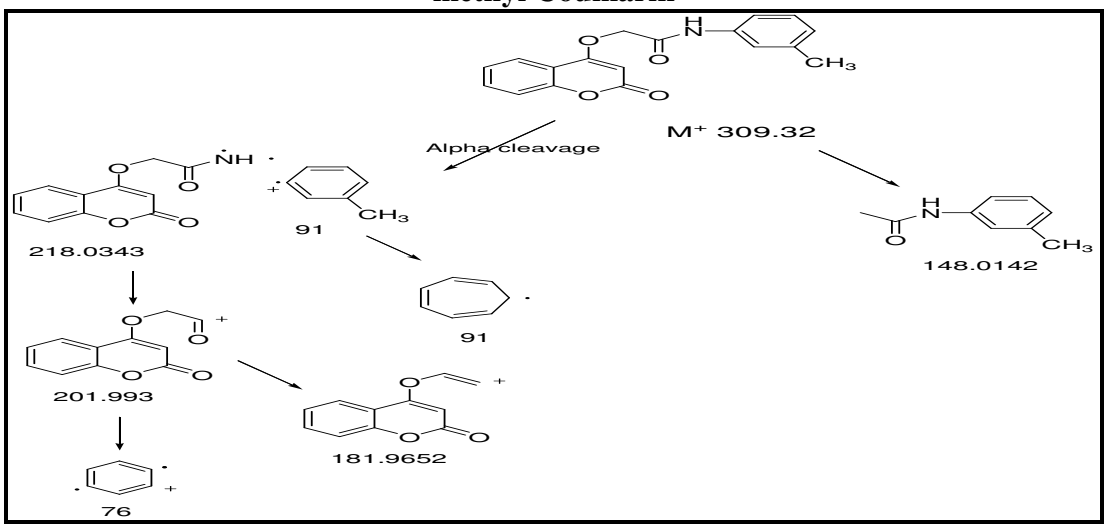
Scheme No.1: Synthetic diagram of 7-Hydroxy-4-Methyl Coumarin

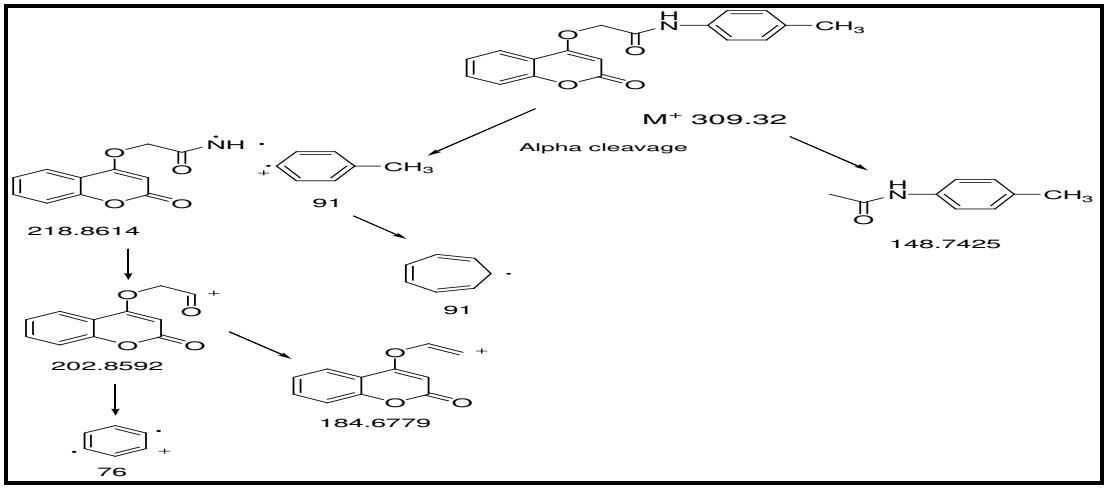


Scheme No.2: Synthetic diagram of Acylated Aromatic amines



Scheme No.3: Synthetic diagram of Coumarin derivatives from 4-hydroxy coumarin and 7-Hydroxy-4-methyl Coumarin





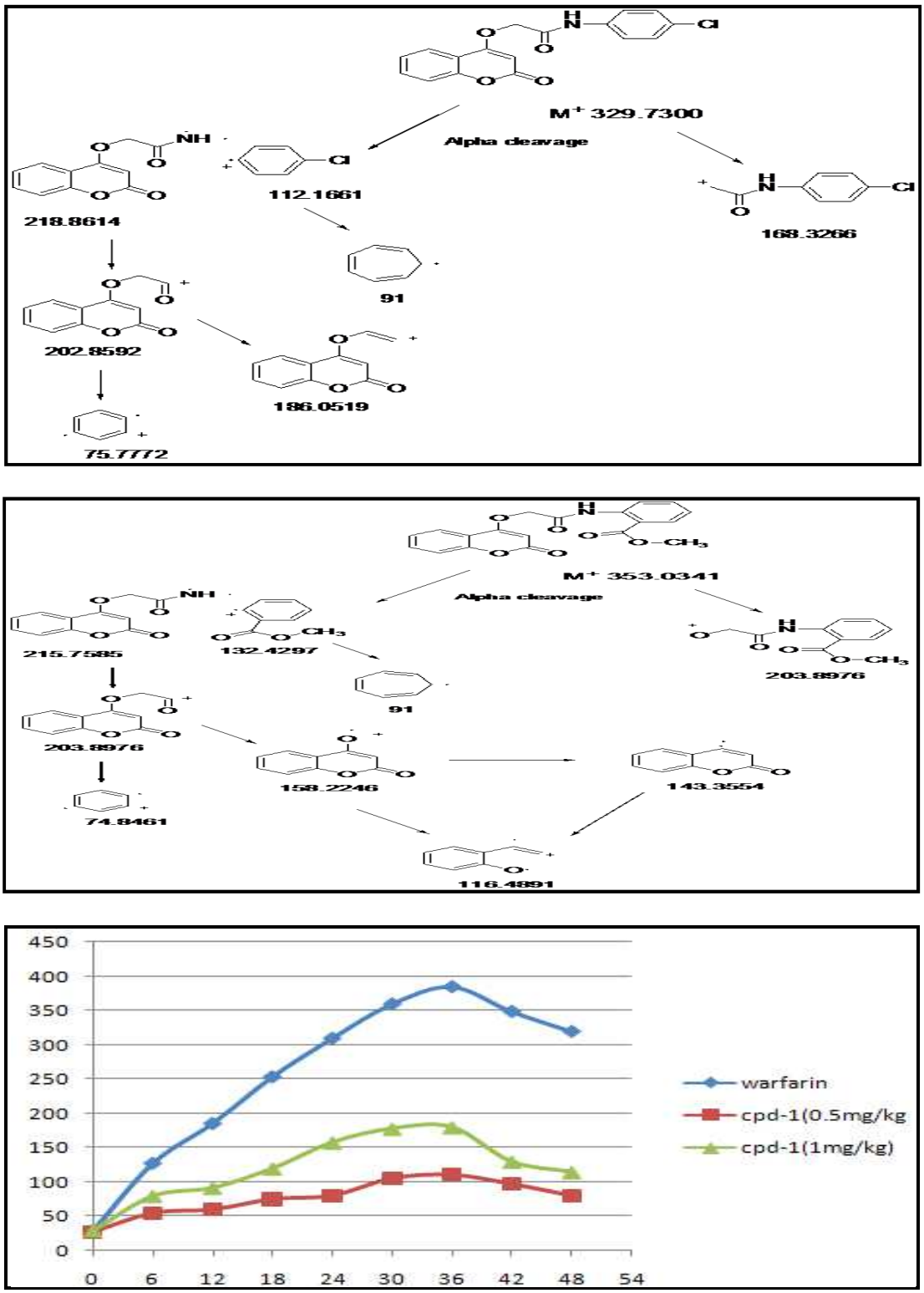


Figure No.1: *In-vivo* anti-coagulant activity data of compound (CPD-1) (capillary tube method of anti-coagulant activity
X-axis=Time intervals of drug administration, Y-axis= Clotting time

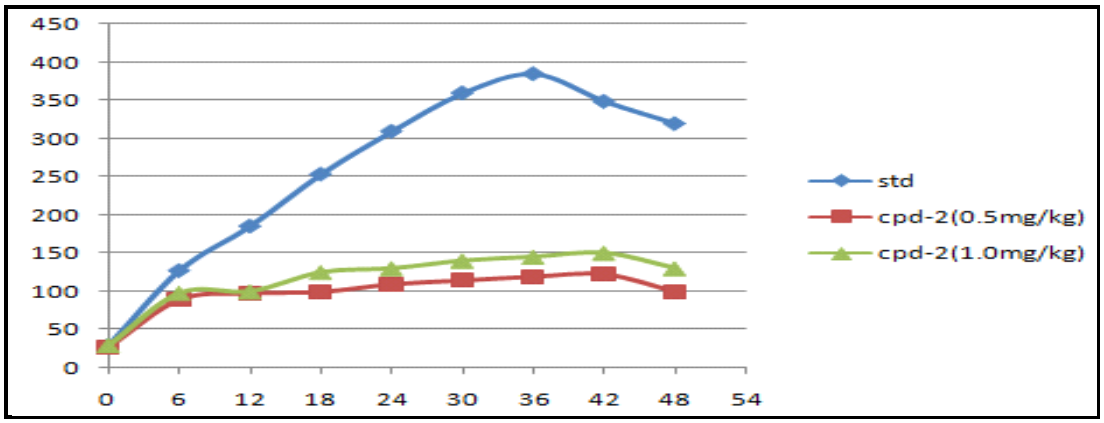


Figure No.2: *In-vivo* anti-coagulant activity data of compound (CPD-2) (capillary tube method of anti-coagulant activity
X-axis=Time intervals of drug administration, Y-axis= Clotting time

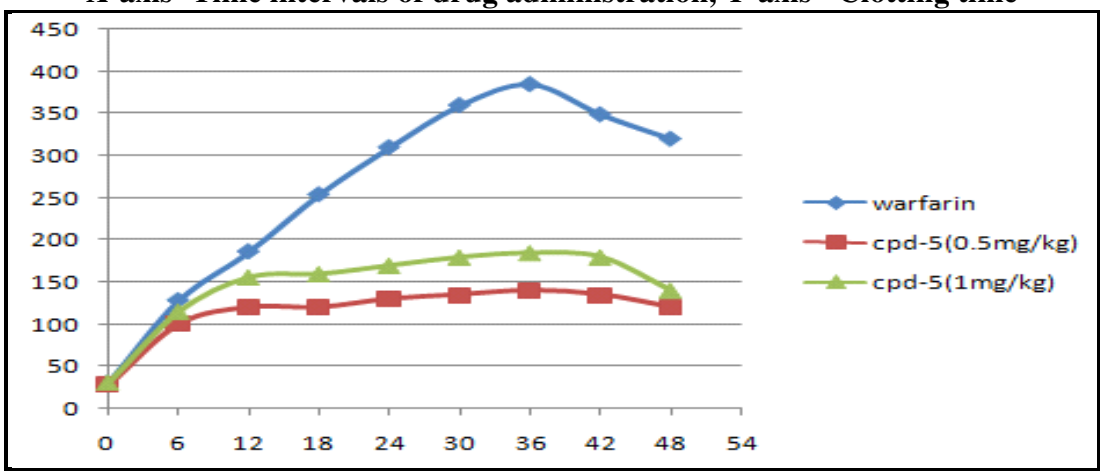


Figure No.3: *In-vivo* anti-coagulant activity data of compound (CPD-5) capillary tube method of anti-coagulant activity
X-axis=Time intervals of drug administration, Y-axis= Clotting time

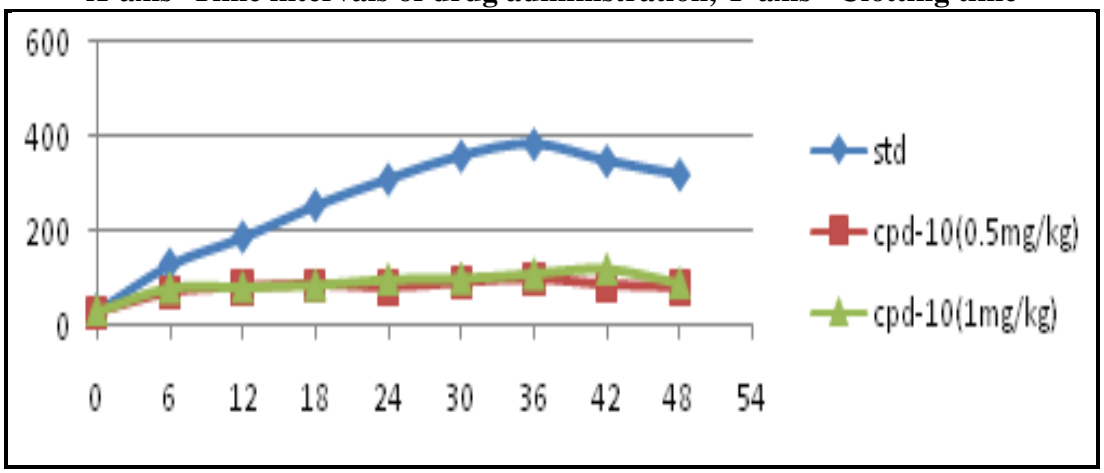


Figure No.4: *In-vivo* anti-coagulant activity data of compound (CPD-10) capillary tube method of anti-coagulant activity.
X-axis=Time intervals of drug administration, Y-axis= Clotting time

SUMMARY

This thesis deals with the investigations carried out on "Design, synthesis and anticoagulant activity of some novel coumarins". The following are some of the findings of the work;

- Considering the structure of warfarin and review of literature some novel coumarins were designed consisting of two series of compounds, one with substitution on the phenolic hydroxyl group of 4-hydroxy coumarin and another series over the phenolic hydroxyl group oh 7-hydroxy-4-methyl coumarin.
- For the synthesis of designed coumarins, acylated amines served as the key intermediate and was prepared from chloroacetylchloride and acetic acid, aromatic amines in two steps. We have synthesized five coumarin derivatives (1 to 5) by using 4-hydroxy coumarin and another five more using 7-hydroxy 4-methyl coumarin.
- All the synthesized compounds were purified by recrystallisation technique.
- The synthesized compounds were characterized by IR, ^{1}H-NMR, ^{13}C-NMR and GC-MS spectra. The IR, ^{1}H-NMR and GC-MS data were consistent with the proposed structures.

In vivo anticoagulant activity of the synthesized compounds was determined by capillary method by taking warfarin as standard anticoagulant on rats. Compounds 1, 2, 5 and 10 exhibited moderate anticoagulant activity, whereas rest of the compounds failed to exhibit anticoagulant activity.

CONCLUSION

A two series of coumarins were designed and synthesized using appropriate synthetic Scheme. The synthesized compounds were purified and well characterized by TLC, IR, ^{1}H-NMR, ^{13}C-NMR and GC-MS data. Compounds CPD-1, CPD-2, CPD-5, CPD-10 are exhibited moderate anti-coagulant activity whereas rest of the compounds failed to show anticoagulant activity.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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