Synopsis

Of

The Thesis Entitled

Studies in synthesis and applications of chromene derivatives

To be submitted to The Maharaja Sayajirao University of Baroda

For the Degree

of

DOCTOR OF PHILOSOPHY

In Chemistry

By

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Under guidance of

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Name of the student: Durgapal Sunil Dutt K

Faculty: Science

Subject: Chemistry

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CHAPTER 1

Introduction to chromene derivatives and their applications

Chromene (Benzopyran) is one of the previleged medicinal pharmacophore which appears as an important structural component in natural products and generated great attention because of their interesting biological activities.

Coumarin belongs to a group of benzopyrones, which consists of a benzene ring joined to a pyrone nucleus. Benzo-α-pyrones (2H-chromen-2-one) commonly known as coumarin, are reported to possess a wide range of biological activities[1-2].

Coumarin (2H-chromen-2-one) and its derivatives are widely distributed in nature[3]. They are regarded as a promising class of bioactive heterocyclic compounds that exhibit wide range of biological activities like anti-microbial, anti-viral, anti-diabetic, anti-cancer, anti-oxidant, anti-convulsant, anti-inflammatory and anti-hypertensive activities etc[4]. In particular, their physiological, bacteriostatic and anti-tumor activities make these compounds attractive backbone for derivatization and screening as novel therapeutic agents. Recently coumarin derivatives have been explored in the field of fluorescence materials and laser dyes[5], nonlinear optical materials[6], photorefractive materials[7].

α,β-Unsaturated ketones, commonly known as chalcones are important class of natural as well as synthetic products which show variety of biological activities. During last few decades, chalcone derivatives have been reported having potent anticancer activity with low side effects and better solubility for therapeutic applications[8-9]. Simple structural modification in chalcone moiety with heterocycles, polyarene compounds or organometal complexes may lead to new anticancer agents with promising activity[10-11]. Chalcone derivatives are also known for their excellent blue light transmittance, good crystallisability[12] and photosensitivity[13]. Photosensitive polymers containing chalcone derivatives have been studied for photoalignment film[14–15].

Cancer is one of the dreadful diseases after cardiovascular diseases all over the world. Most of the cancers are defined by uncontrolled growth of cells without differentiation due to the deregulation of essential enzymes and other proteins controlling cell division and proliferation[16-17]. Out of many therapeutic strategies, chemotherapy shows significant clinical responses. At the same time, these chemotherapeutic agents have a small therapeutic window

with non-specificity and high-systemic toxicity. To get selective chemotherapeutics with very low side effects is a major challenge in treatment of cancer[18].

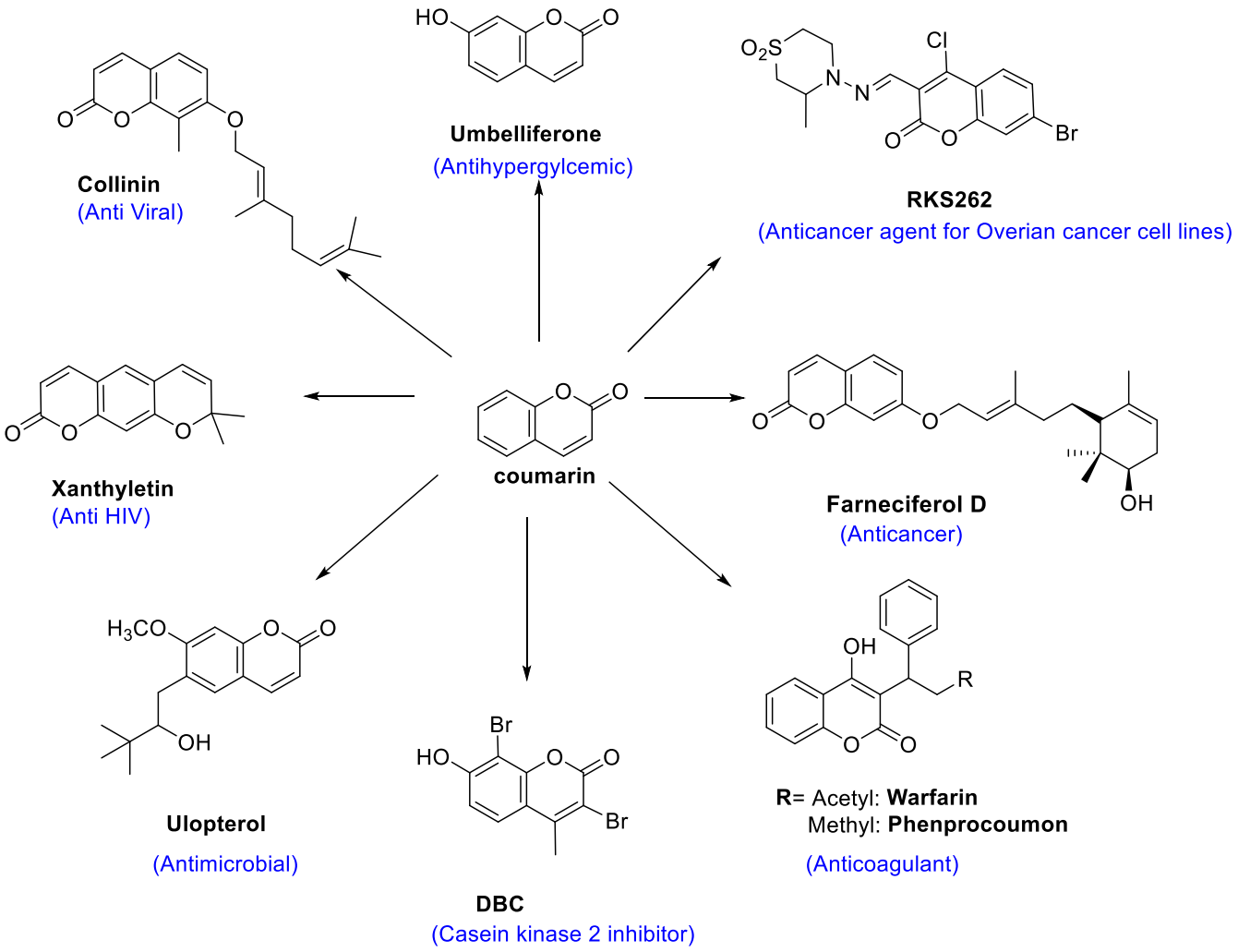


Figure 1: Some biologically active Chromene-2-one derivatives

In coumarin compounds, the studied properties are fluorescence, colouring agents, liquid crystalline and gelation behaviour in water and organic solvents. These properties received special attention because they are considered as promising candidates for the next generation of materials, due to their dynamic response, environmental compatibility and low energy processing with non-covalent interactions to form organized soft materials[19].

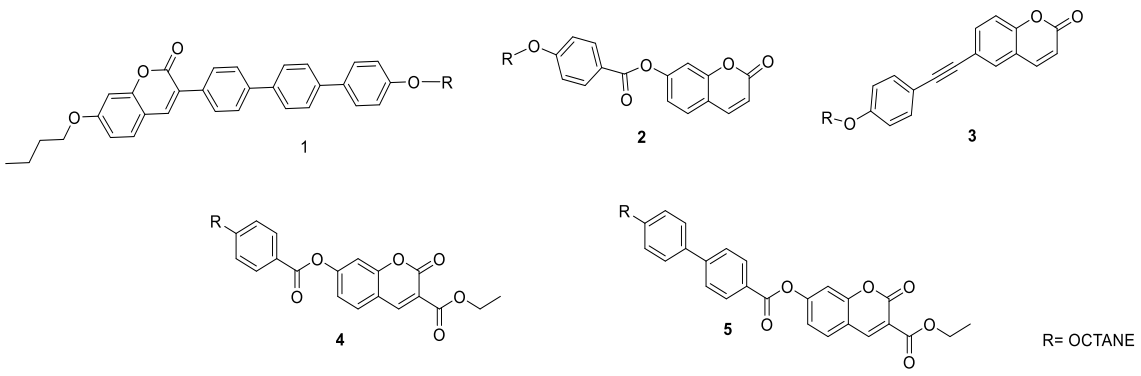


Figure-2: Coumarin derivatives with liquid crystalline properties

Various coumarin derivatives showing mesomorphic state are reported (figure-2). Ethyl 7-hydroxycoumarin-3-carboxylate derivatives showed excellent liquid crystal behavior majorly Smectic-A and nematic phase[20].

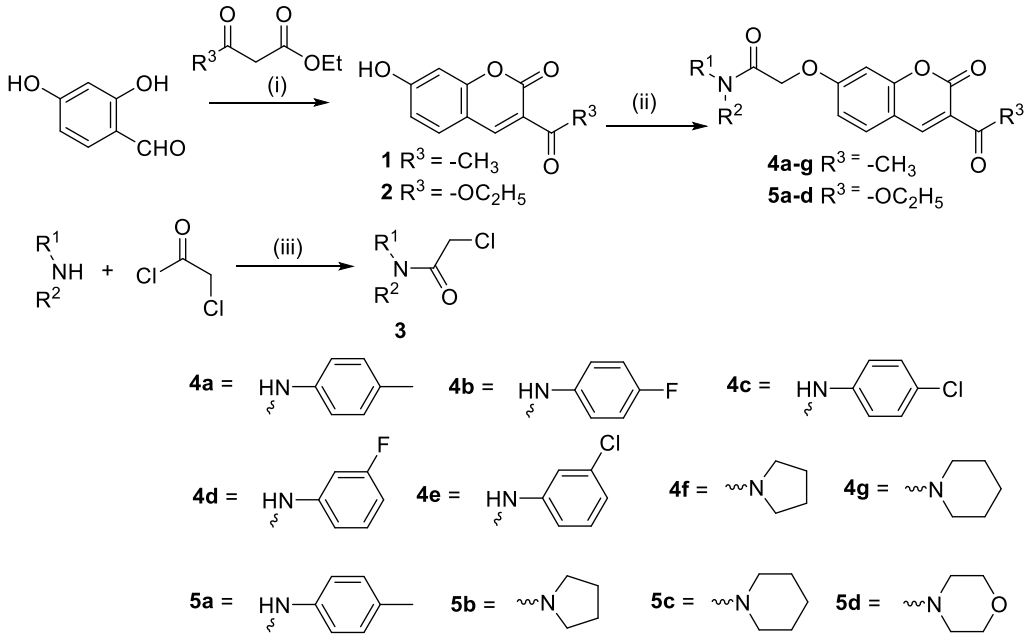
CHAPTER 2

Synthesis and application of 3,7-disubstituted benzopyrone derivatives as anticancer agent.

Chapter 2 is divided into two series, in series-1 synthesis of 3-acetyl 7-hydroxy coumarin is carried out and then various amide derivatives of it were synthesized as shown in scheme-1 (compounds 4a-g). In 2nd series, 3-carboxylate 7-hydroxy coumarin was prepared and then its various amide derivatives were synthesized as shown in scheme-1 (compounds 5a-d) [21]. All the compounds were characterized by using different spectral techniques like ^{1}H NMR, ^{13}C NMR, IR, ESI-MS and C,H,N analysis

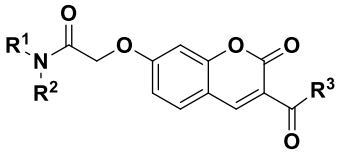
Compounds **4a-g** and **5a-d** were screened for their anticancer activity by using MTT assay method in lungs (A549) and breast cancer (MCF7) cell lines. Compound **4b** showed excellent anticancer activity against A549 cell line with IC_{50} 0.16 nM. Compound **4b** and **4e** were studied further for its binding with CT-DNA by UV and Fluorescence spectroscopy

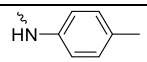
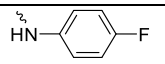
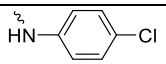
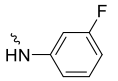
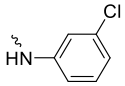
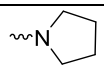
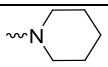
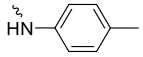
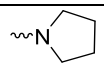
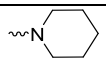
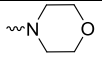
Scheme-1



Reagents and conditions: (i) Piperidine catalytic, pyridine, bulb oven (100 W), 70-80 ^{o}C, 14 h; 74–87% (ii) **3**, anhydrous K_2CO_3, KI pinch, DMF, 70-80 ^{o}C, 12-18 h; 43–91% (iii) TEA, DCM, 0-5 ^{o}C, 30 min, rt, 24 h. 85–95%

Table 1: Anticancer activity against A549 (Lungs cancer cell line) and MCF7 (Breast cancer cell line) **4a-g** and **5a-d**.



Compd no.	NR^1R^2	R^3	A549 IC_{50}	MCF7 IC_{50}
4a		$-CH_3$	2.40 µM	0.65 µM
4b		$-CH_3$	0.16 nM	23.53 µM
4c		$-CH_3$	0.82 µM	13.02 µM
4d		$-CH_3$	9.16 µM	14.04 µM
4e		$-CH_3$	89.16 µM	84.8 nM
4f		$-CH_3$	23.9 µM	3.08 µM
4g		$-CH_3$	5.06 µM	1.11 µM
5a		$-OC_2H_5$	NA	1.78 µM
5b		$-OC_2H_5$	3.11 µM	0.79 µM
5c		$-OC_2H_5$	NA	NA
5d		$-OC_2H_5$	23.2 µM	21.61 µM
5-Fluorouracil			11.13 µM	45.04 µM

[a]IC_{50} values were determined using Graph Pad Prism software by MTT assay using DMF. NA = Not active

Table 2: Anti-oxidant activity of compounds **4a-e** and **5b-c**.

Compoud no.	EC_{50} µg/mL[a]
4a	3436
4b	882
4d	48
4e	59
5b	46
5c	47
Ascorbic acid	11

[a]EC_{50} values were determined using Graph Pad Prism software by DPPH assay using DMF.

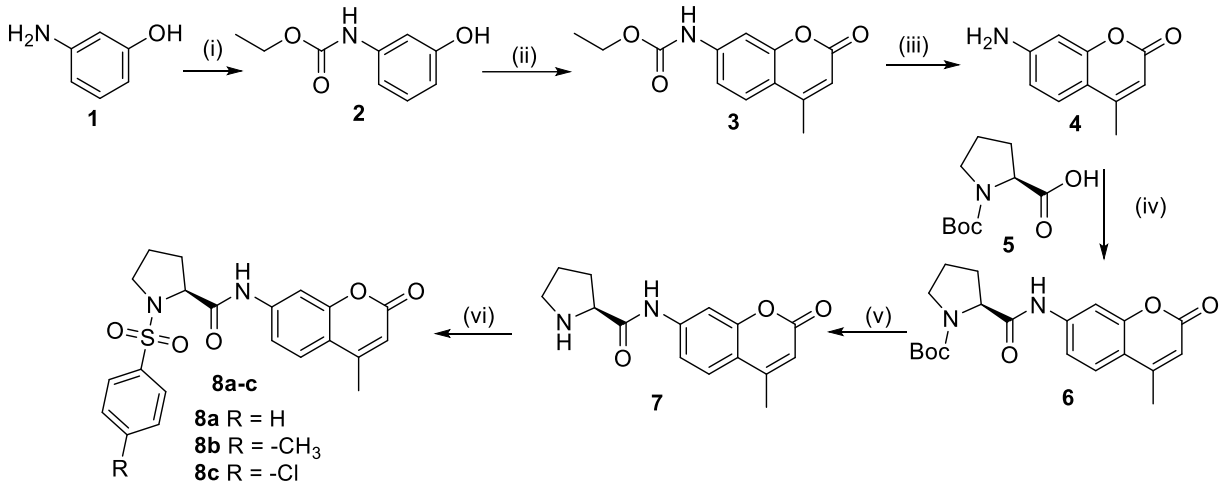
CHAPTER 3

3A: Synthesis of chromene-2-one proline-sulphonamide hybrids for antidiabetic and anticancer evaluation

Chapter 3A deals with preparation of proline sulphonamide hybrid derivatives of 7-amino-4-methyl-2H-chromen-2-one (**4)** (scheme-1), 3-amino-2H-chromen-2-one **(13)** (scheme-2) and 2-amino-3H-benzo[f]chromen-3-one (**14)** (scheme-2)**.** The amino group was further reacted with Boc-proline to give tert-butyl (S)-2-((4-methyl-2-oxo-2H-chromen-7-yl)carbamoyl)pyrrolidine-1-carboxylate (**6)**. Then Boc group was removed by stirring in 10% trifluoro acetic acid (TFA) in dichloro methane (DCM) to give compound **7**. Compound **7** on reaction with substituted benzene sulphonyl chloride gave the desired coumarin proline hybrids **8a-c**. All the intermediates and final compounds were characterized by using different spectral techniques like ^{1}H NMR, ^{13}C NMR, IR, ESI-MS and C,H,N analysis for all compounds.

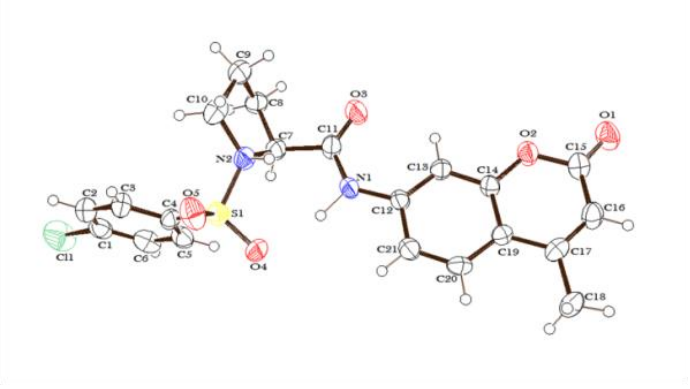
The anticancer activity of **8a-c**, **19a-c** and **20a-c** was studied by MTT assay in lungs and breast cancer cell line and antidiabetic activity by DPP-4 inhibition assay.

Scheme-1

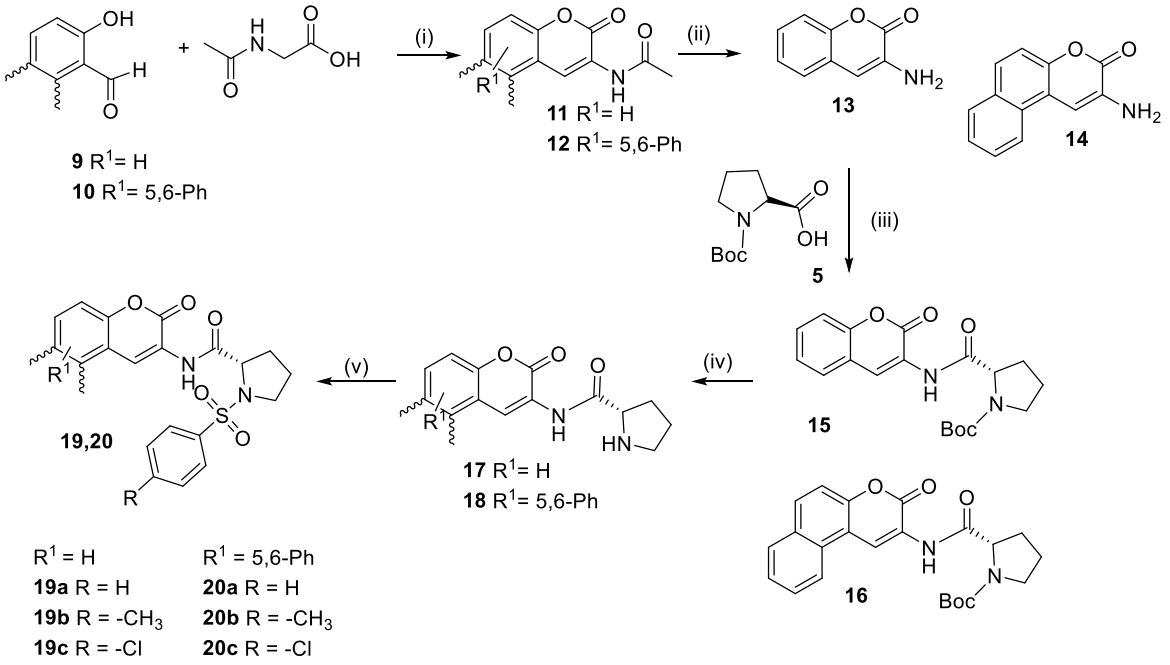


Reagents and conditions: (i) CH_3CH_2OCOCl, ethyl acetate, rt; 85% (ii) CH_3COCH_2COOEt , H_2SO_4:C_2H_5OH (3:7), rt; 55% (iii) H_2SO_4:CH_3COOH (1:1), reflux; 42% (iv) (a) THF, CH_3CH_2OCOCl (0-5 C) 10 mins (b) Boc proline, THF, TEA reflux 8h. 75-85% (v) TFA, DCM, rt; 90-95% (vi) $NaHCO_3$, benzeneulphonyl chloride, DCM:Water(1:1), rt. 85-87%

The structure of **8c** was also confirmed by its single crystal analysis with CCDC number 1876142.

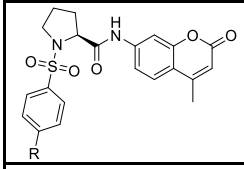
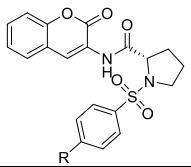
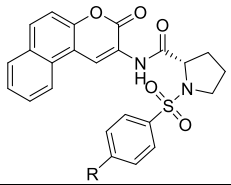


Scheme-2



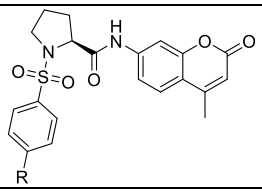
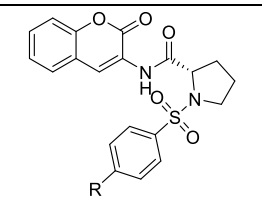
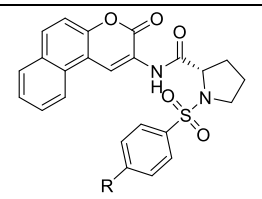
Reagents and conditions:(i)NaOAc, Ac_2O, reflux, 4h; 64% (ii) Conc HCl : EtOH (7:3), reflux; 52% (iii) (a) THF, CH_3CH_2OCOCl (0-5 C) 10 mins (b) Boc proline, THF, TEA reflux 8h; 75-80% (iv) TFA, DCM; 95% (v) $NaHCO_3$, benzenesulphonyl chloride, DCM:Water(1:1).85-95%

Table 1: DPP-IV inhibition activity of amino coumarin-proline sulphonamide derivative **8a-c**, **19a-c** and **20a-c**

Structure	Compds	R	% DPP-4 enzyme inhibition activity[a]		
			25 µM	50 µM	100 µM
	**8a**	-H	11.11	16.29	19.36
	8b	-CH3	5.05	7.54	13.96
	8c	-Cl	10.90	17.08	20.76
	**19a**	-H	9.87	13.60	20.17
	19b	-CH3	13.76	19.36	20.73
	19c	-Cl	13.04	18.67	22.00
	**20a**	-H	7.06	10.44	14.32
	20b	-CH3	9.12	12.90	16.02
	20c	-Cl	11.68	15.31	19.79
	Vildagliptin		56.3 % at 0.1µM		

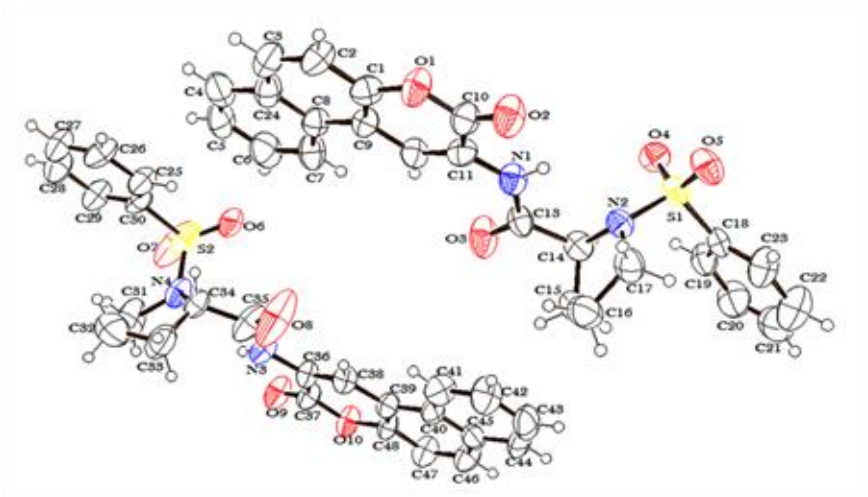
[a]DPP-IV inhibitory activity determined by fluorescence-based assay was measured using Spectra Max fluorometer (Molecular Devices, CA). Values of % inhibition are mean of three independent determinations at 25, 50 and 100 µM concentrations of the test samples.

Table 2 : Anticancer activity against A549 (Lungs cancer cell line), MCF-7 (Breast cancer cell line) for coumarin-proline sulphonamide hybrid derivatives

Compound	R^1	R	IC_{50} in µM[a]	
			A549	MCF-7
7			2.34	5.42
8a		H	18.09	2.58
8b		$-CH_3$	27.54	**1.07**
8c		-Cl	27.32	7.05
17			12.40	17.14
19a		H	9.34	4.39
19b		$-CH_3$	N.A	7.759
19c		-Cl	7.39	3.81
18			3.75	11.74
20a		H	19.95	22.94
20b		$-CH_3$	11.95	7.37
20c		-Cl	23.09	50.99
Fluorouracil			11.13	45.04

[a]IC_{50} values were determined using Graph Pad Prism software by MTT assay using DMF. NA = Not active

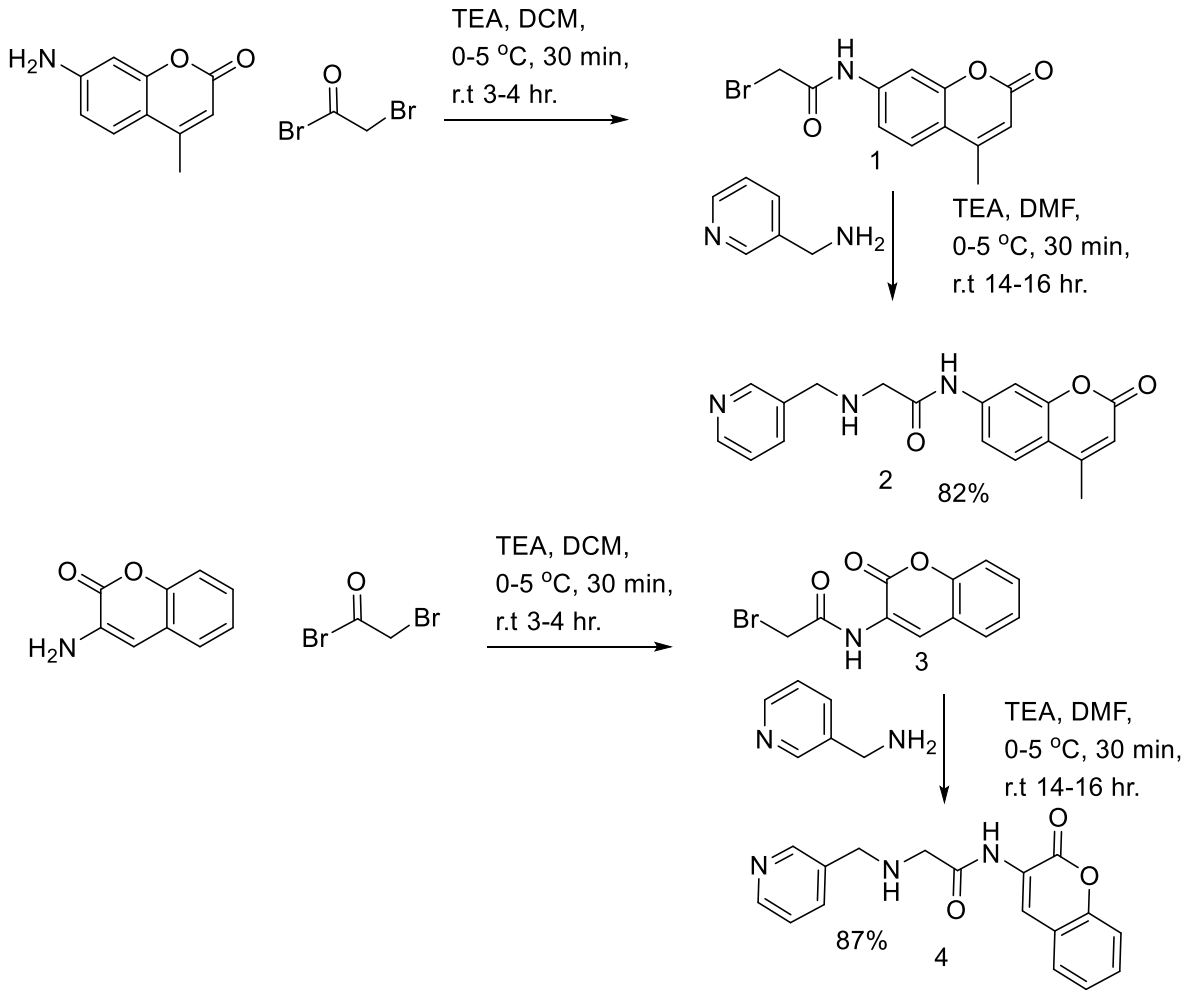
The structure of **20a** was also confirmed by its single crystal analysis with CCDC number 1876145



3B: Synthesis and Cytotoxic studies of chalcone derivatives from 3-aminomethyl pyridine.

In this chapter I have prepared 7-amino 4-methyl coumarin and 3-amino coumarin, which on reaction with bromoacetyl bromide gave compound 2-bromo-N-(4-methyl-2-oxo-2H-chromen-7-yl)acetamide (**1)** and 2-bromo-N-(2-oxo-2H-chromen-3-yl)acetamide (**3).** Compound **1** and **3** on reaction with 3-amino methylpyridine gave compound **2** and **4** as shown in scheme-1.

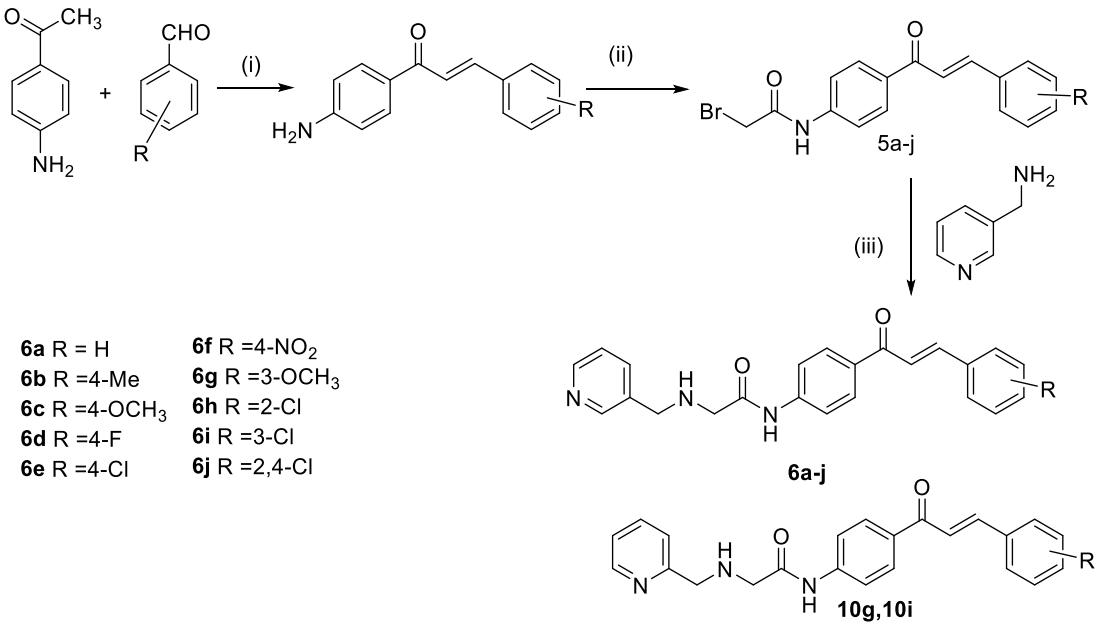
Scheme 1



p-Amino acetophenone on reaction with various aldehydes in the presence of base gave chalcone **3,** (scheme-2) which on reaction with bromoacetyl bromide gave compound **4**. Compound **4** on reaction with 3-aminomethyl pyridine gave compounds 6a-j and 2-methylaminopyridine gave compounds 10g, 10i as shown in scheme-2. All newly synthesized compounds were characterized by using different spectral techniques like ^{1}H NMR, ^{13}C NMR, IR, ESI-MS and elemental analysis. Compounds **6g** and **6i** gave excellent anticancer

activity by MTT assay in lung and breast cancer cell lines[22]. Both compounds **6g**, **6i** showed good binding constant Kb. LDH and EtBr/AO assay confirmed cell death by apoptosis pathway.

Scheme 2



Reagents and conditions: (i) 20% aq NaOH, ethanol, rt, 5-10 h; (ii) bromoacetyl bromide, TEA, DCM, rt, 16-18 h; (iii) TEA, DMF, 0-5 ^{o}C, 30 min, rt , 16 h, 34-76%

Table-1 Anticancer activity against A549 (Lungs cancer cell line), MCF-7 (Breast cancer cell line) for compounds **2, 4, 6a-j 10g, 10i**.

Compd no	R	IC_{50} in µM[a]		Compd no	R	IC_{50} in µM[a]	
		A549	MCF-7			A549	MCF-7
2	-	177.10	377.8	**4**	-	69.1	298.9
6a	-H	6.18	53.27	**6g**	3-OMe	32.42	0.174
6b	4-Me	132.00	90.61	**6h**	2-Cl	46.89	71.72
6c	4-OMe	0.269	n/a	**6i**	3-Cl	0.245	6.7nM
6d	4-F	16.04	n/a	**6j**	2,4-Cl	62.26	92.21
6e	4-Cl	5.14	n/a	**10c**	4-OMe	36.25	13.07
6f	$4\text{-}NO_2$	28.19	n/a	**10i**	3-Cl	10.32	16.25
Fluorouracil		11.13	45.05				

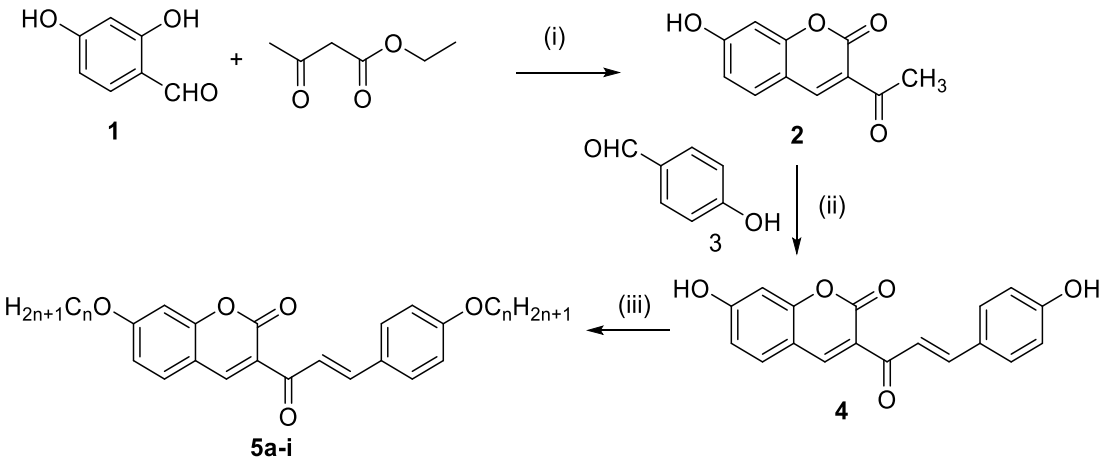
[a]IC_{50} values were determined based on MTT assay using GraphPad Prism software.

CHAPTER 4

4A: Synthesis of chromene-chalcone derivatives and its applications as liquid crystals.

This chapter deals with synthesis of chalcone derivatives. Resorcaldehyde on reaction with ethyl acetoacetate gave 7-hydroxy-3-acetyl coumarin **2** which on reaction with p-hydroxy benzaldehyde gave chalcone **4** as shown in scheme-1. The free hydroxyl group at 7th position of coumarin and 4th position of benzene ring in compound **4** was further alkylated with different alkyl halides to give compounds **5a-i**. All newly synthesized compounds **5a-i** were characterized by using different spectral techniques like ^{1}H NMR, ^{13}C NMR, IR, ESI-MS and elemental analysis, Transition temperatures for all compounds were recorded by Differential scanning calorimetry. Liquid crystalline phase study of all synthesized compounds was studied by Polarizing optical microscope. Compounds showed mesogenic properties such as nematic and smectic phase

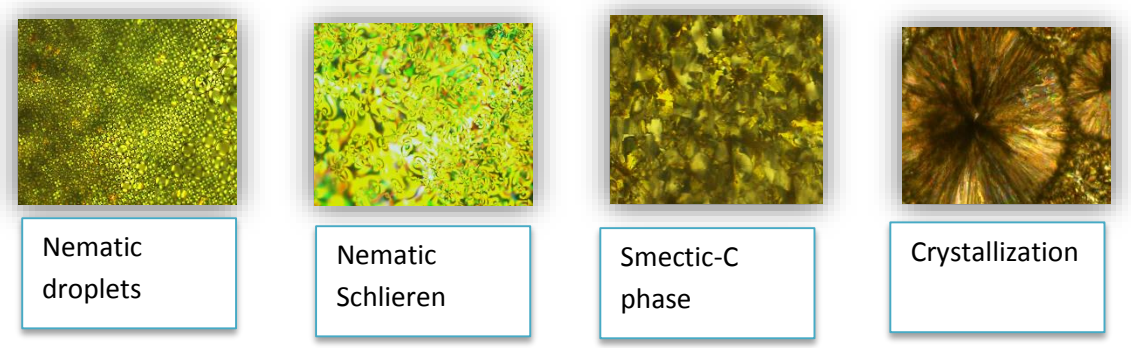
Scheme-1



n = 2, 4, 6, 8, 10, 12, 14, 16, 18

Reagents and conditions: (i) ethyl acetoacetate, ethanol, piperidine, reflux,16 h, 72-85%; (ii) 4-hydroxy benzaldehyde, ethanol, pyrrolidine, acetic acid, reflux, 16 h, 40-45% (iii) dry K_2CO_3, DMF, alkyl halide, 12h reflux, 80-85%

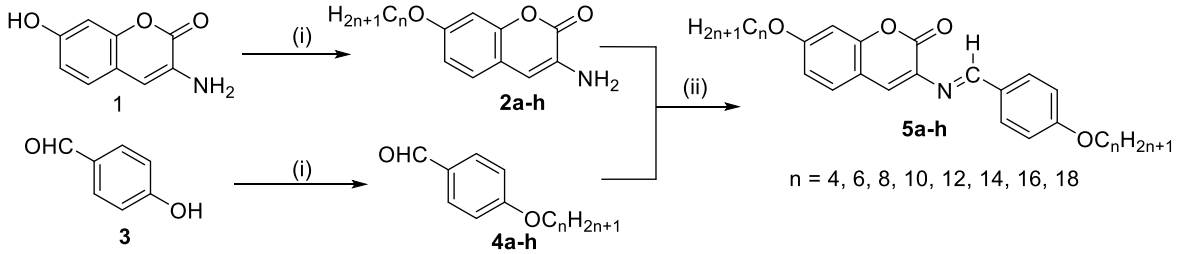
Figure-1: Polarizing optical microscope images on cooling cycle of Chalcones **5d.**



4B: Synthesis of imine linked chromene derivatives and its applications as liquid crystal.

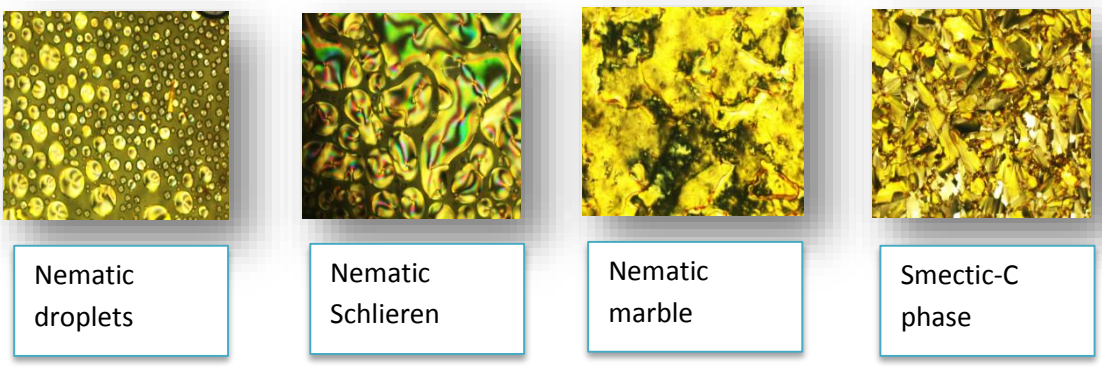
3-Amino 7-hydroxy coumarin **1** on reaction with various alkyl halides in presence of base like K_2CO_3 gave 7-alkoxy-3-amino coumarin **2a-h** as showin in scheme-1. p-Hydroxy benzaldehyde 3 on reaction with various alkyl halides gave 4-alkoxy benzaldehydes (4a-h) which on reaction with various 7-alkoxy-3-amino coumarin (2a-h) in presence of catalytic amount of acetic acid gave schiff bases 5a-h. These new imine derivatives 5a-h were characterized by various spectral techniques like ^{1}H MNR, ^{13}C NMR, IR, ESI-MS and elemental analysis, DSC studies were carried out and the mesogenic properties were also studied using Polarizing optical microscope. The compound showed various phase such as nematic and smectic as shown in fig-1

Scheme-1



Reagents and condition: (i) K_2CO_3, DMF, alkyl Bromide, 18-20 h, rt; (ii) ethanol, catalytic acetic acid, reflux, 16-18 h. 76-90%

Figure-2: Polarizing optical microscope images on cooling cycle cycle of Imine **5c**.

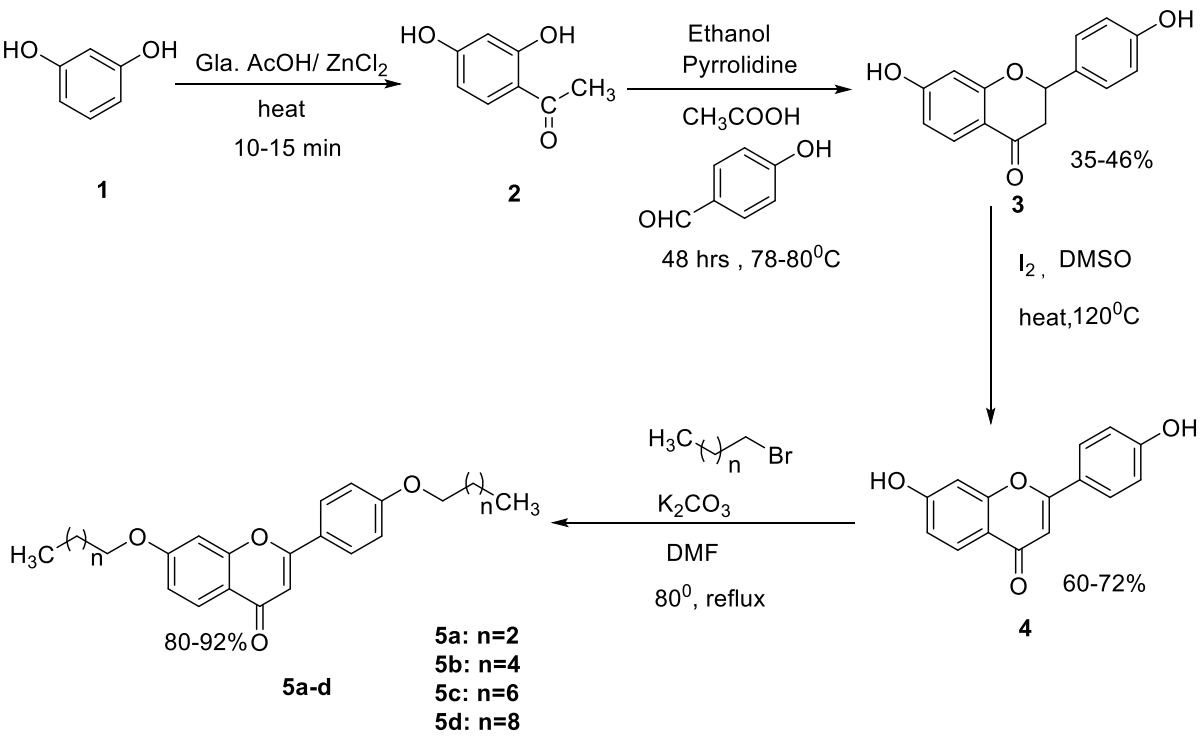


CHAPTER 5

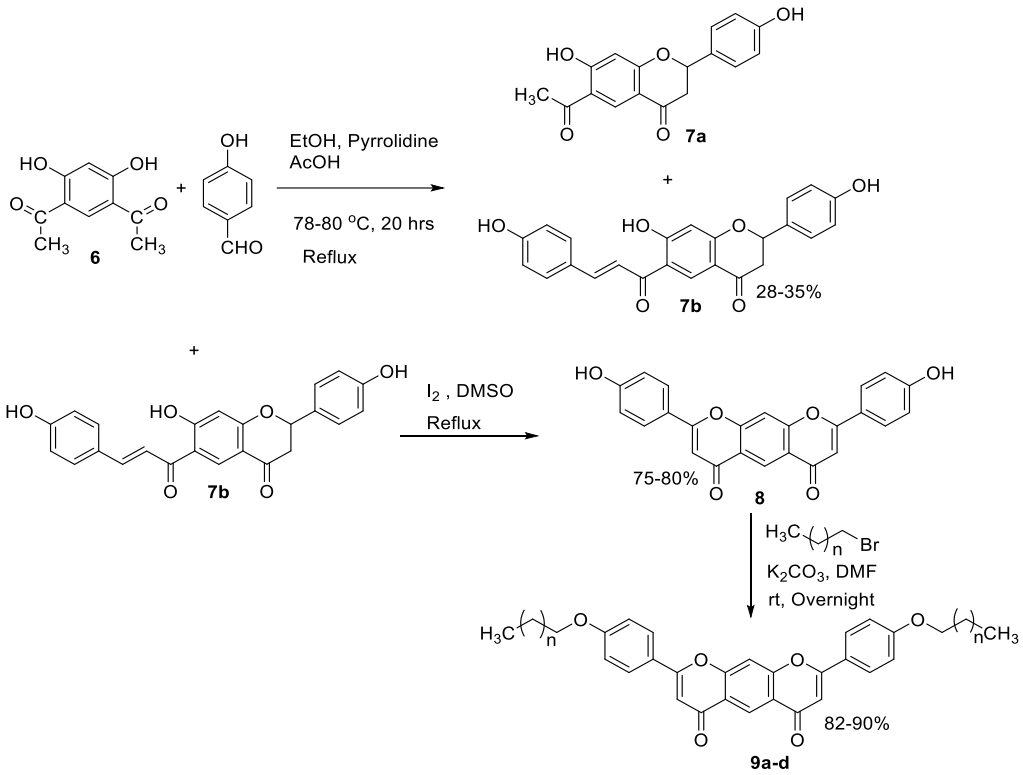
Synthesis of chromene-4-one and bis chromene-4-one derivatives and its applications

Resacetophenone on reaction with p-hydroxy benzaldehyde in presence of base like pyrrolidine gave dihydroflavone **3** which was dehydrogenated in presence of I_2, DMSO to give flavone **4**. The free hydroxyl groups in compound **4** were alkylated using various alkyl halides in presence of base like K_2CO_3 to give desired flavones **5a-d** scheme-1. Similarly bis flavones were synthesized by starting with 2,4-dihydroxy-1,5-diacetyl benzene, as shown in scheme-2. The structures of all the newly synthesized compounds were confirmed by various spectral techniques like ^1H NMR, ^{13}C NMR, IR, ESI-MS. The mesogenic properties of these compounds were studied using polarizing optical microscope, but unfortunately not a single compound showed liquid crystalline property.

Scheme-1



Scheme-2



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