Synthesis, Characterization of Biodynamic Heterocyclic system of 3-Acetyl Coumarins by Knoevenagel condensation reaction and acetylation of coumarin with study of Biological Screening

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Abstract
*Ecofriendly, solvent free,conveniently synthetic procedure adopted to synthesise substituted 3-acetyl coumarin was afforded via Knoevenagel condensation reaction(KCR). The spontaneous cyclization of 3-methoxy salicyldehyde and 4-*diethylamino *salicyldehyde with β–ketoester (ethylacetoester) possessing active methylene group using piperidine in pyridine as a catalyst yielded heterocyclic 8-methoxy-3-acetyl--2H-chromen-2-one (HAC) and 3-acetyl-7-(diethyl amino)-2H-chromen-2-one (DAC) respectively.*

Another synthesis is by direct acetylation of 7-hydroxy coumarin and 4-methyl 7-hydroxy coumarin using piperidine in pyridine catalyst to form 7-hydroxy-3-acetyl--2H-chromen-2-one (HAC) and 7-hydroxy-4-methyl-3-acetyl-2H-chromen-2-one (HMAC). These four products formed with high acceleration reaction rate, high yield show (solvatochromic)superb optical properties and dynamic biological activities.

Keywords: Acetyl Coumarin, Knoevenagel condensation reaction, Acetylation, Biological Screening.

Introduction

Coumarins is the vernacular name of the tonka bean (Dipteryx odorata Wild, Fabaceae family) from which coumarin was isolated in 1820. There are four major class of coumarin: the simple coumarins, furanocoumarins, pyranocoumarins and the pyrone-substituted coumarins. The sources of coumarins at high levels are in some essential oils in cinnamon bark oil (7,000 ppm), cassia leaf oil (up to 87,300 ppm) and lavender oil. Coumarin is also found in fruits (e.g. bilberry, cloudberry), green tea and other foods such as chicory.[1]

The π- expanded coumarins have been used in various research areas due to their unique photophysical properties[2,3]. The various π -expanded coumarins have been prepared and studied[4]. π- expanded coumarins possessing additional benzene or heterocyclic rings fused in different ways with chromen-2-one were synthesized by Pechmann and Walsk[5] in 1884 and π -expanded chromophores attracted attention due to the natural occurrence of various derivatives especially possessing due to skeleton of benzo[c]coumarin and shifted in the last few years towards cutting-edge optoelectronic applications[6].

Diversity-oriented syntheses are highly advantageous for exploring structural and functional characteristics leading to a heterogenic product library[7]. The photo physical properties and electronic structure of 2-substituted 3-ethynylquinoxalines synthesized from electron rich π nucleophiles, oxalyl chloride, terminal alkynes and 1,2-diaminoarenes are studied.

The obtained compounds are highly fluorescent with remarkable emission solvatochromism[8]. A straight forward, regioselective and step-economical ligand-free palladium-catalysed decarboxylative functionalization of coumarin-3-carboxylic acids is devised. This protocol is compatible with a wide variety of electron-donating and withdrawing substituents and allows for construction of various biologically important π-electron extended coumarins[9], biomedical imaging agents[10,11], potential use as light emitters in organic light-emitting diodes,therapeutic agents[12].

Fig. 1: 3D Structure of MAC

Material and Methods

3-methoxy salicyldehyde (99%), 4-diethylaminosalicylaldehyde (99%), 7-hydroxycoumarin(98%),7-hydroxy-4-methyl coumarin (97%), ethyl acetoacetate (99+%), piperidine (99%) chemicals were purchased from Alfa Aesar (Great Britain) and pyridine (99%) and acetyl chloride (97%), DMF (97%), DMSO (97%) from S.D Fine Chemicals, AR grade and used without purification. Ethanol, methanol, acetone, n-hexane and ethyl acetate were purified on rotavapor.

Fig. 2: 3D Structure of DAC

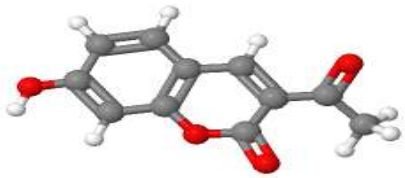


Fig. 3: 3D Structure of HAC

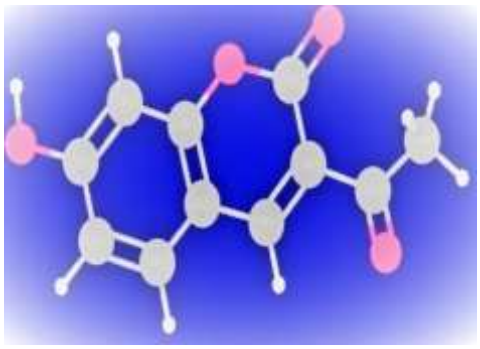


Fig. 4: 3D Structure of HMAC

Measurements: IR spectra were recorded on Bruker FT-IR Spectrophotometer Alpha-II having Platinum ATR single reflection diamond ATR moduled (without KBr) at Research Lab, Chemistry Department, Sant Gadge Baba Amravati University, Amravati (M.S.). Elemental analysis was carried out at CHN analyzer at SAIF Chandigarh (Punjab).^{1}H and ^{13}C spectra were recorded on Bruker advance Neo and Mass spectra on mass spectrophotometer at SAIF -Panjab University, Chandigarh. UV-Visible absorbance spectra were recorded in DMSO solvent on Shimadzu (double beam) at Central Instrumentation Centre (CIC) at Shri Shivaji Science College, Amravati.

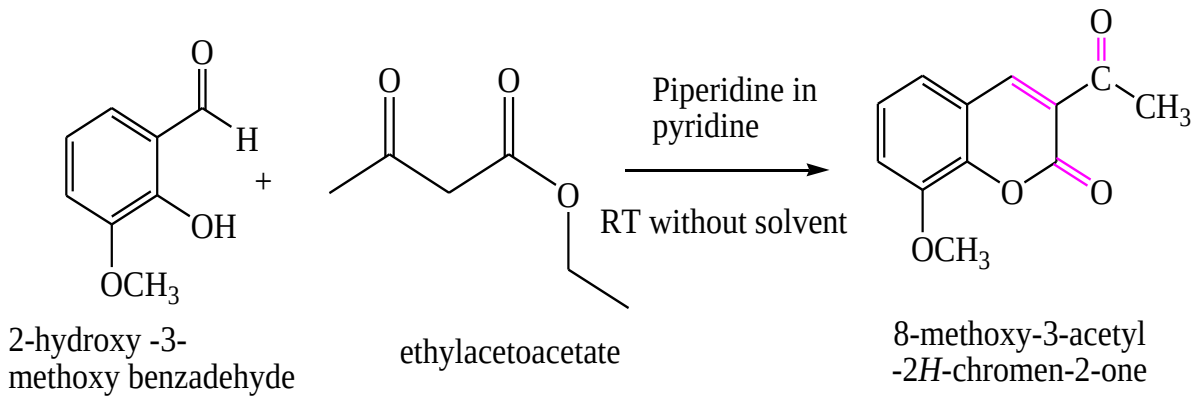
Experimental

Synthesis of 3-acetyl coumarin by Knoevenagel Condensation Reaction (KCR)

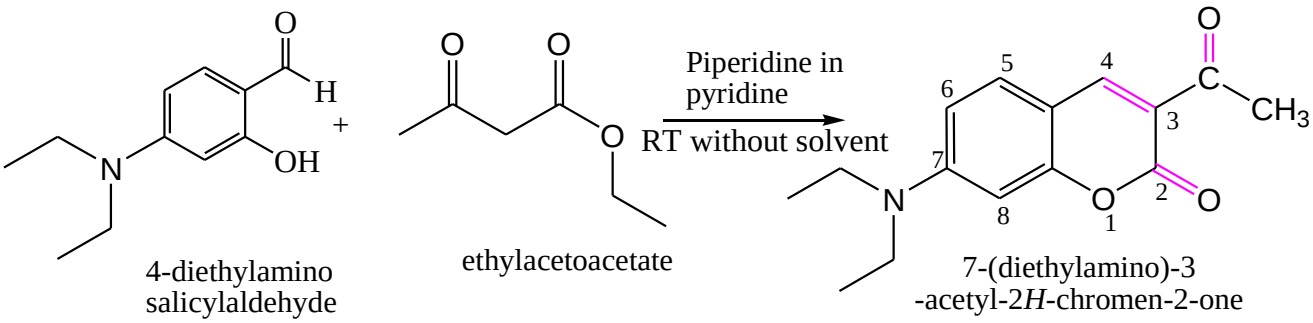
1. Synthesis of 8-methoxy-3-acetyl--2H-chromen-2-one (MAC): - In a round bottom flask, equimolar quantities of 0.0125 mole (1.90 gram) 2-hydroxy 3-methoxy salicylaldehyde were thoroughly mixed with ethyl acetoacetate 0.0125 mole (1.75 gram) with addition of 0.5 ml piperidine in pyridine (1:1) as a base catalyst as shown in scheme 1. The reaction mixture was stirred for 10 minutes without addition of any solvent.

The reaction was monitored by TLC using 7:3 proportions of ethyl acetate and n-hexane as a solvent system. The faint yellow vigorous solution was formed and neutralized with 1M HCl. The product was recrystallized from dry ethanol. The yield of pure product was 1.52gm (94%). M.P. was determined with open capillary on classic electrical m. p. apparatus. The structure of coumarins was confirmed by CHN analyzer, spectral physical tools, IR, ^{1}H-NMR, ^{13}C-NMR and HR-Mass Spectroscopy.

2. Synthesis of 7-(diethyl amino) 3-acetyl -2H-chromen-2-one (DAC): In the round bottom flask, equimolar quantities of 0.0125 mole (2.415gram) of 4-diethyl amino salicylaldehyde were thoroughly mixed with ethyl acetoacetate 0.0125 mole (1.932ml) with addition of 0.5 ml piperidine in pyridine (1:1) as a base catalyst. The reaction mixture was stirred for 10 minutes without addition of any solvent. The reaction was monitored by TLC using 7:3 proportions of ethyl acetate and n-hexane. The bright yellow vigorous solution was formed and neutralized with 1M HCl. The product was collected by suction filtration recrystallized from dry ethanol. The yield of pure product was 1.94gm (82.55%). Melting point was determined with open capillary on classic electrical melting point apparatus. The structure of coumarins was confirmed by CHN analyzer, spectral physical tools, IR, ^{1}H-NMR, ^{13}C-NMR and HR-Mass Spectroscopy.



Scheme 1: Synthesis of MAC



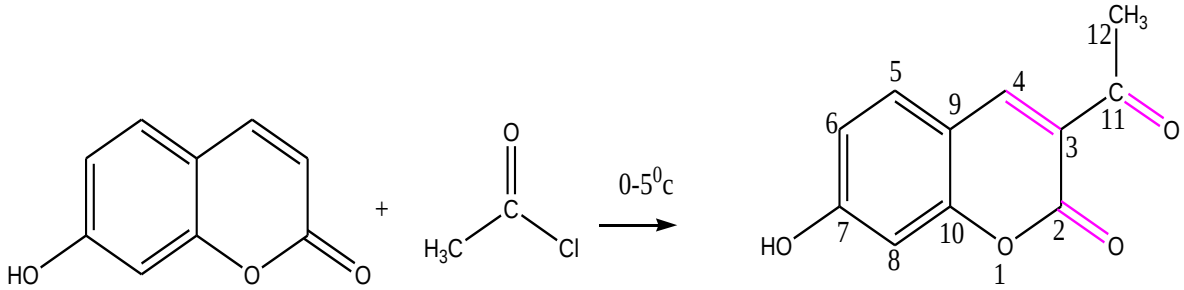
4-diethylamino salicylaldehyde

ethylacetoacetate

7-(diethylamino)-3 -acetyl-2*H*-chromen-2-one

Scheme 2: Synthesis of DAC

3. Synthesis of 7-hydroxy-3-acetyl--2H-chromen-2-one (HAC) by direct acetylation: In two necked round bottom flask, the equimolar quantities of 0.0125 mole (2.02 gram) of 7-hydroxy coumarin (umbelliferrone) was dissolved in minimum quantity of dry MeOH and added slowly to 0.0125mole (0.981ml) acetyl chloride with constant stirring using 0.5 ml piperidine in pyridine (1:1) as a base catalyst.[24]. The reaction was exothermic, temperature of reaction mixture was maintained between 0-5^{0}C. The reaction mixture was refluxed for 30 minutes. The reaction was monitored by TLC using 70:30 proportion ratio of ethyl acetate and n-hexane. The brown colour product was obtained and neutralized with 1M HCl. The product was collected by suction filtration and recrystallized from dry ethanol to afford the compound. Melting point was determined with open capillary on classic electrical melting point apparatus. The yield of pure product was 1.90 gm (%).



7-hydroxy-2*H*-chromen-2-one

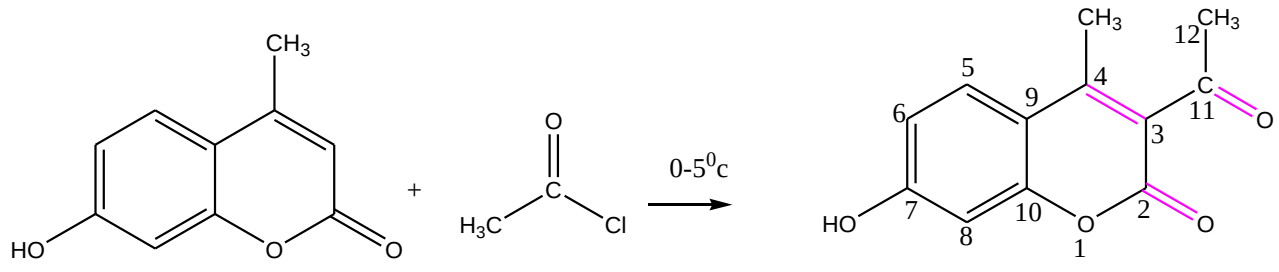
acetyl chloride

3-acetyl-7-hydroxy-2*H*-chromen-2-one

Scheme 3: Synthesis of HAC

4. Synthesis of 7-hydroxy-4-methyl-3-acetyl-2H-chromen-2-one (HMAC) by direct acetylation: In two necked round bottom flask the equimolar quantities of 0.0125 mole (2.2 gram) of 7-hydroxy -4-methyl coumarin (hymercromone) were dissolved in minimum quantity of dry MeOH and added slowly to 0.0125mole (1.08ml) acetyl chloride with constant stirring using 0.5 ml piperidine in pyridine (1:1) as a base catalyst. The reaction was exothermic, temperature of reaction mixture was maintained between 0-5^{0}C. The reaction mixture was refluxed for 30 minutes. The reaction was monitored by TLC using 70:30 proportion ratio of ethyl acetate and n-hexane. The brown colour product was obtained and neutralized with 1M HCl. The product was collected by suction filtration and recrystallized from dry ethanol to afford the compound. The yield of pure product was 1.1 gm (67.48%).

Melting point was determined with open capillary on classic electrical melting point apparatus. The structure of coumarins was confirmed by spectral analytical techniques-Elemental analysis by CHN analyzer, IR, ^{1}H-NMR, ^{13}C-NMR and HR-Mass Spectroscopy.



7-hydroxy-4 methyl-2*H*-chromen-2-one

acetyl chloride

7-hydroxy-4 methyl-3-acetyl-2*H*-chromen-2-one

Scheme 4: Synthesis of HMAC

Table 1
Micro analytical data of substituted derivatives of 3-acetyl 2-*H*-chromene-2-one

Coumarin	Mol. Formula	Mol.wt. g.mol^{-1}	Colour	Elemental		Solubility	M.P.^{o}C	Yield %
				Found %	Calc%			
MAC	($C_{12}H_{10}O_4$)	218.20	Yellow	C, 66.0 H,4.61	C,65.98 H,4.51	EtOH,DMF DMSO	178-180	94
DAC	($C_{15}H_{17}O_3N$)	259.30	Bright yellow	C,69.47 H, 6.6 N,5.4	C,69 H,6.3 N,5.1	EtOH, DMF, DMSO	152-154	82.55
HAC	($C_{11}H_8O_4$)	204.17	brown	C, 64.70 H,3.94	C, 64 H,3.50	EtOH,DMF DMSO	170-172	90
HMAC	($C_{12}H_{10}O_4$)	218.20	Light yellow	C, 66.05 H,4.61	C,65 H,3.68	EtOH,DMF DMSO	100-102	67.48

Table 2
FT-IR spectra (cm^{-1}) data of substituted derivatives of 3-acetyl 2-*H*-chromene-2-one

Coumarins	Vibrational Frequencies data (cm^{-1})
MAC	1727 (ν C=O lactone), 1335(ν C=C Ar),1679 (ν C=O ketone), 1564(νC=C Ar), 1469(νC=C ethylenic), 1601(νC-HAr), 1356(νC-C acetyl), 1092(ν C-O methoxy).
DAC	1717(ν C=O lactone), 1659 (ν C=O ketone), 1612 (ν C=C Ar), 1565 (ν C=O Methoxy),1501(ν C=H Ar), 1345(ν C-N amine), 2960(νC-C, CH3-C, SP^3).
HAC	1716 (ν C=O lactone), 2960w(νOH-Phenolic),1658(νC=O ketone),1611(C=C Ar),1564 (νC=C ethylenic), 1501 (νC=H Ar).
HMAC	3120b(νOH-Phenolic),1672 (ν C=O lactone), 1588 (ν C=O ketone),1381 (ν C=C ethylenic).

Table 3
^{1}H spectral data (ppm) of substituted derivatives of 3-acetyl 2-*H*-chromene-2-one

Types of Coumarins	(δ in ppm 500MHz DMSO-d_6)
MAC	3.31(3H, s,-OCH_3), 2.57(3H,s,$COCH_3$-H), 8.59(1H,s, ethylenic), 7.30(1H,t,Ar-H), 7.41, 7.47 (2H,dd,Ar-H).
DAC	1.15(6H,t,two of CH_3 in amine), 3.51(4H,q,two of CH_2 in amine),2.51(3H,s,OCH_3-H), 7.66 (1H,d,Ar-H),6.67 (1H,d,Ar-H), 6.5 (1H,s,Ar-H), 8.48(1H,s,-ethylenic).
HAC	2.52(3H, s, methyl), 7.95(1H,s,ethylinic), 10.59(1H,s,Ar-OH), 6.80 -7.50 (3H,s,d-d,Ar-H).
HMAC	2.37(3H, s, 4-methyl), 6.83(1H,d,Ar-H), 6.73(1H,s,Ar-H), 7.5(1H,d,Ar-H), 2.5(3H,s, OCH_3), 10.55(1H,s,Ar-OH).

Results and Discussion

Micro analytical data: The synthesised coumarins are coloured and insoluble in water, but soluble in ethanol, DCM, warm DMF and DMSO. The % values of element are found in agreement with calculated values. The mol.wt was calculated using standard procedure. Melting point was determined repeatedly in open capillary on melting point apparatus.

FT-IR Spectra: The IR spectra (table 1) of all coumarins showed characteristics of (ν C=O lactone) at 1727, 1717,1716 cm^{-1}. A high intensity band at C=O of ketone at 1679,1659,1658,1588 cm^{-1} is observed in four type of coumarins, 1345(ν C-N amine), the band at 1345 cm^{-1} due to C-N in amine, The band appears of ethylenic C=C at 1449,1381,1564 cm^{-1}.The absorption of (νOH-Phenolic) was observed in HAC and HMAC at 2960,3120 cm^{-1} characteristics of -OH group in this compound. FT-IR spectra are given in figures 5 to 8.

^{1}H spectra: The spectrum of ^{1}H NMR in DMSO-d_6 was recorded at 500MHz in ambient temperature. The coumarins MAC, DAC, HAC, HMAC of sharp singlet at δ 3.31, 2.51, 2.5 suggest the methyl group. The multiplate,d-d and singlet observed shows the aromatic ring current of proton lies at δ 7.30-6.5 in all cases.

The phenolic hydrogen clearly represented high splitting value at δ 10.59,10.55 in third and fourth coumarin. The triplet and quartet at 1.15 and 3.51-CH_3 and -CH_2 group are

observed in 7-diethylamino-3-acetyl coumarin. The singlet suggested ethylenic proton at 4th position >CH=C< at spectral value of δ 8.59, 8.48, 7.59 in all cases. ^{1}H spectra are given in figures 9 to 12.

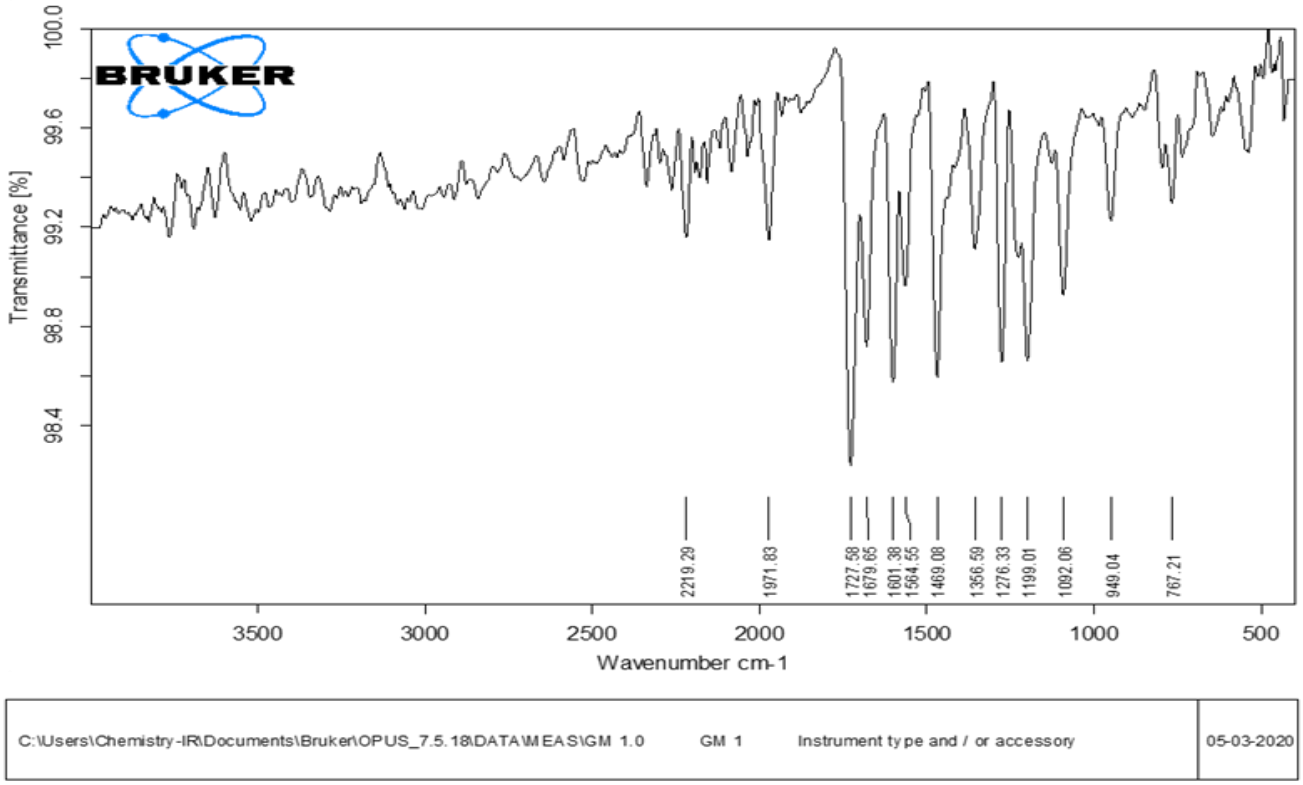


Fig. 5: IR spectrum of 8-methoxy-3-acetyl--2H-chromen-2-one (MAC)

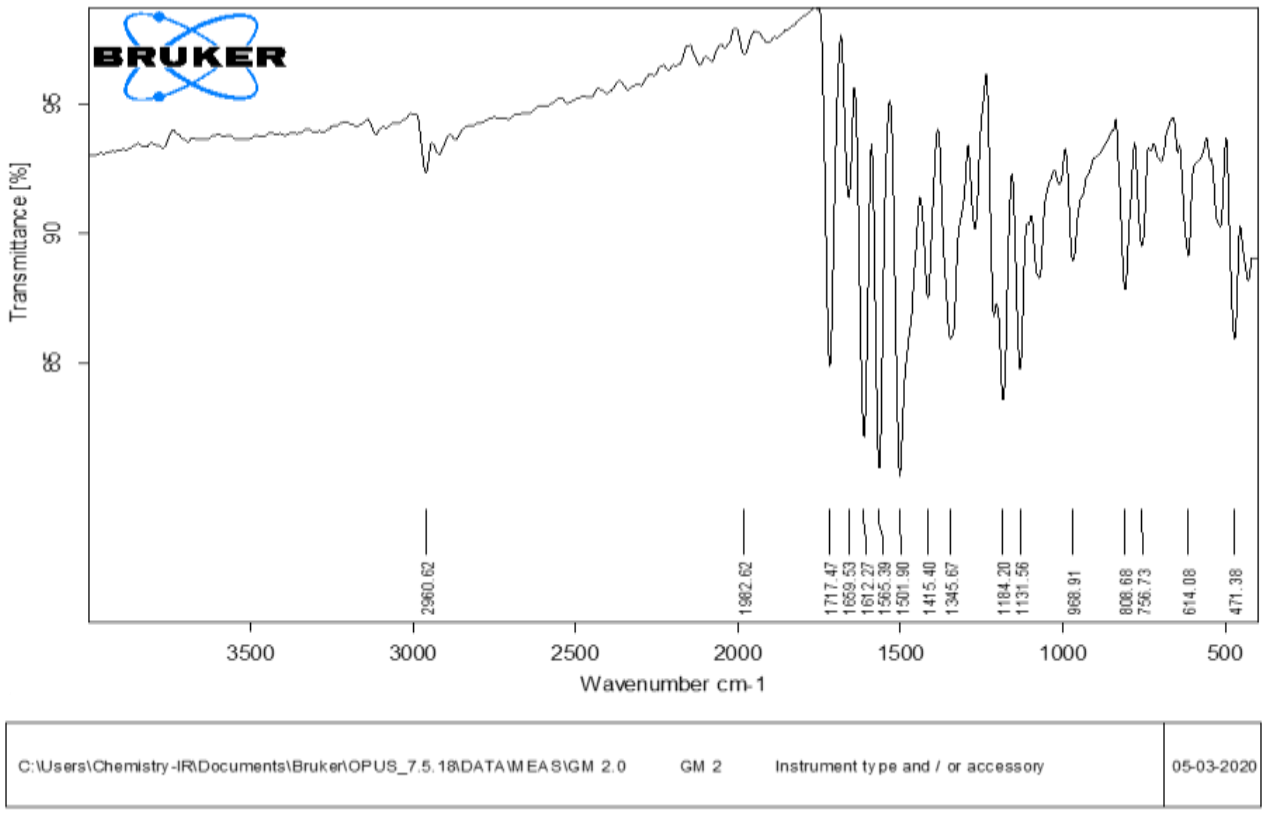


Fig. 6: IR spectrum of 7-(diethyl amino)- 3-acetyl-2H-chromen-2-one (DAC)

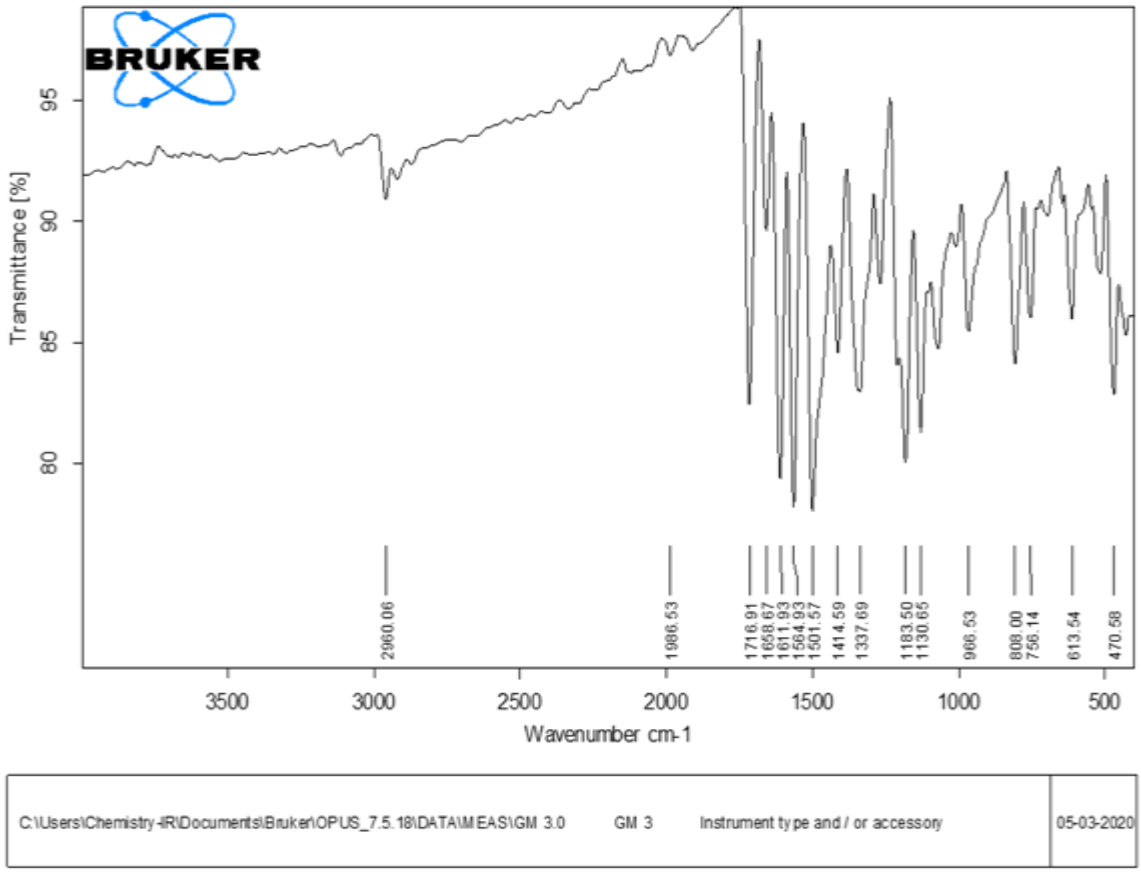


Fig. 7: IR spectrum of 7-hydroxy-3-acetyl--2H-chromen-2-one (HAC)

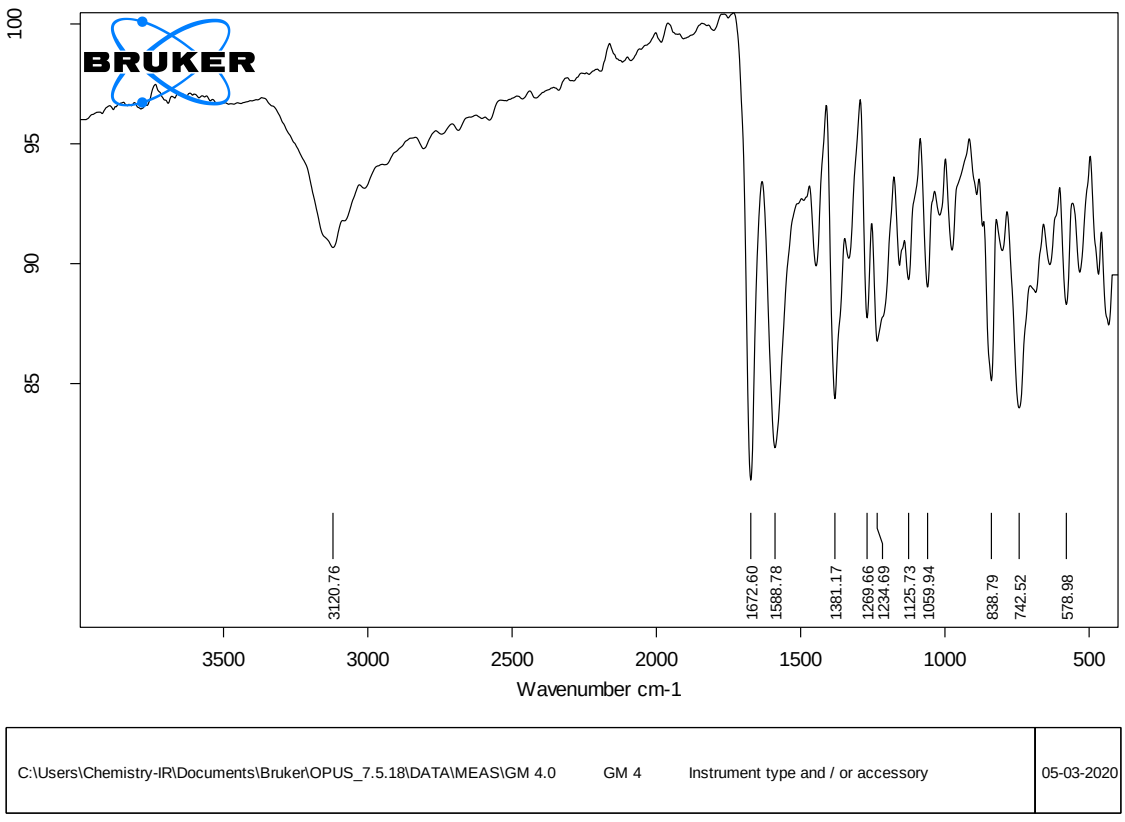


Fig. 8: IR spectrum of 7-hydroxy-4methyl-3-acetyl--2H-chromen-2-one (HMAC)

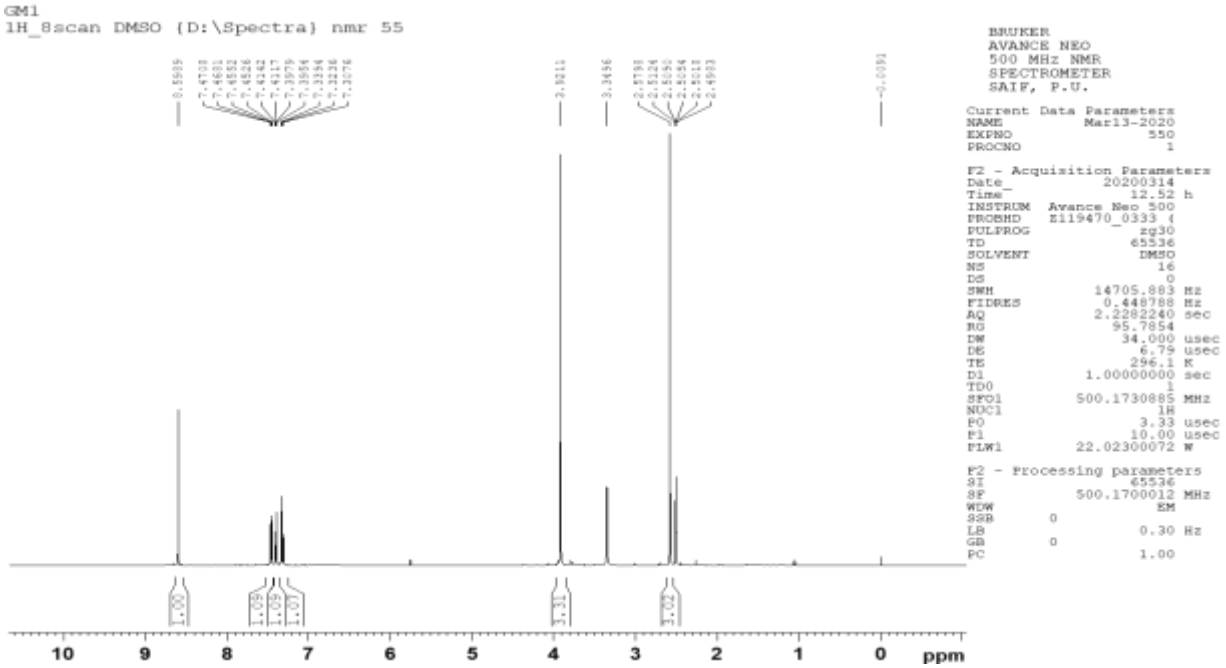


Fig. 9: ^{1}H spectrum of 8-methoxy-3-acetyl--2H-chromen-2-one (MAC)

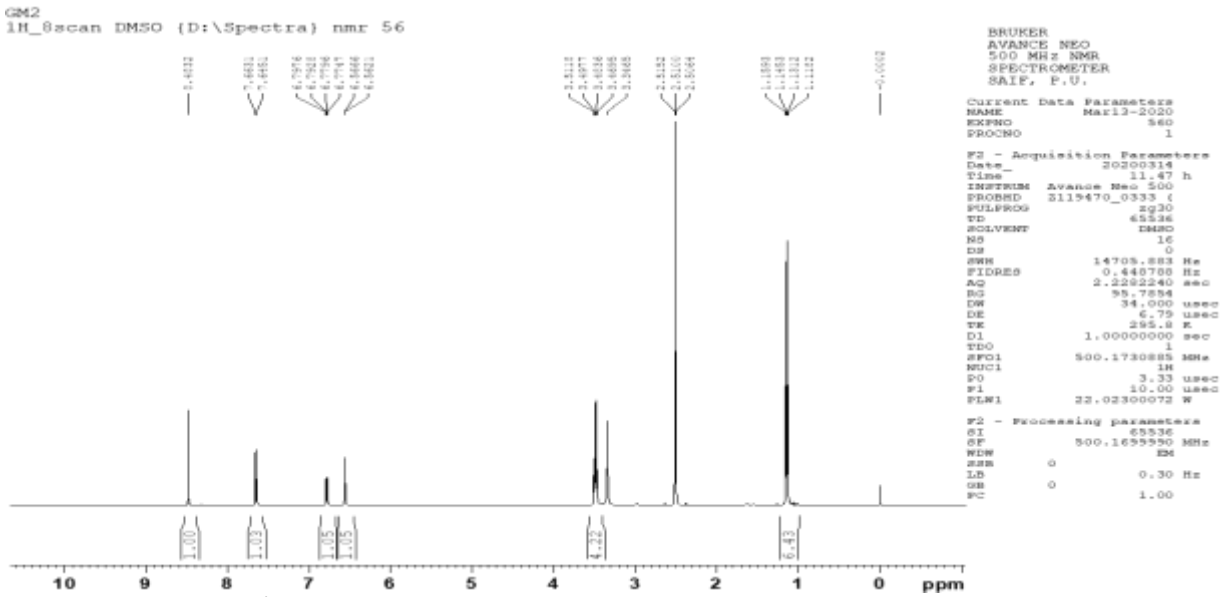


Fig. 10: ^{1}H spectrum of 7-diethylamino-3-acetyl--2H-chromen-2-one (DAC)

Table 4
^{13}C-NMR spectral data (ppm) of substituted derivatives of 3-acetyl 2-*H*-chromene-2-one

Coumarin Compound		**^{13}C (δ in ppm 500MHz DMSO-d_6)**
MAC	($C_{12}H_{10}O_4$)	29.90(CH_3),194.95(C=O acetylenic),158(C=O lactone),124 (C3), 143(C4), 121(C5), 124(C6), 116(C7) ,147(C8), 118(C9), 143(C10), 56.05 (C11, C13).
DAC	($C_{15}H_{17}O_3N$)	159.78(C2 lactone),132.32(C3),147(C4),132(C5),111.8(C6),152(C7), 107(C8),147(C9),110(C10),44.31(C11),12.23(C12),44.31(C13),12.23(C14),194.09 (C15, C=O ketone),30.04(C16)
HAC	($C_{11}H_8O_4$)	161.19(C2, C=O lactone),129.59(C3),144(C4),111.29 (C6), 160(C7,C-OH), 102.05(C8,Ar-C),113(C9, Ar-C), 155(C10,Ar-C).
HMAC	($C_{12}H_{10}O_4$)	160(C2,C=O,lactone),126.36(C3),159(C4),126.36(C5,Ar-C), 110(C6,Ar-C),161.11(C7,Ar-C), 111.93(C8,Ar-C),102.14(C9,Ar-C), 153.30.14(C10,Ar-C),18.01(C13-CH_3).

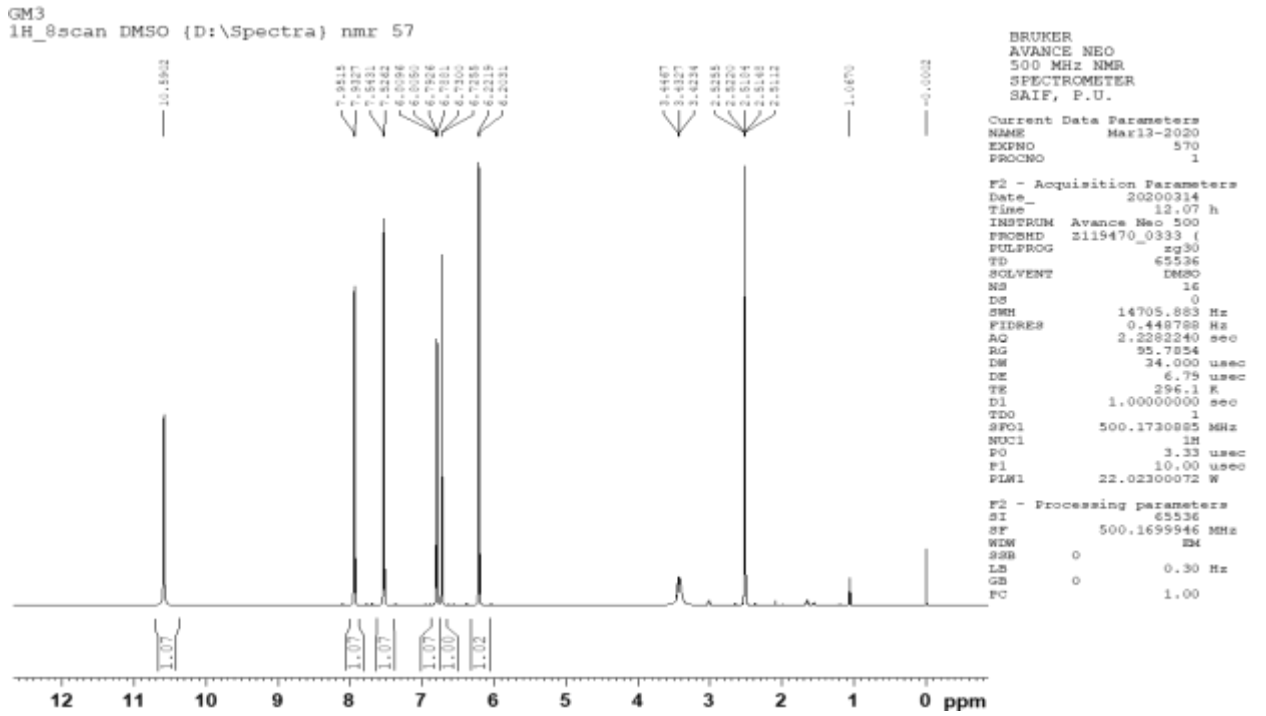


Fig. 11: 1H-NMR spectrum of 7-hydroxy-3-acetyl--2H-chromen-2-one (HAC)

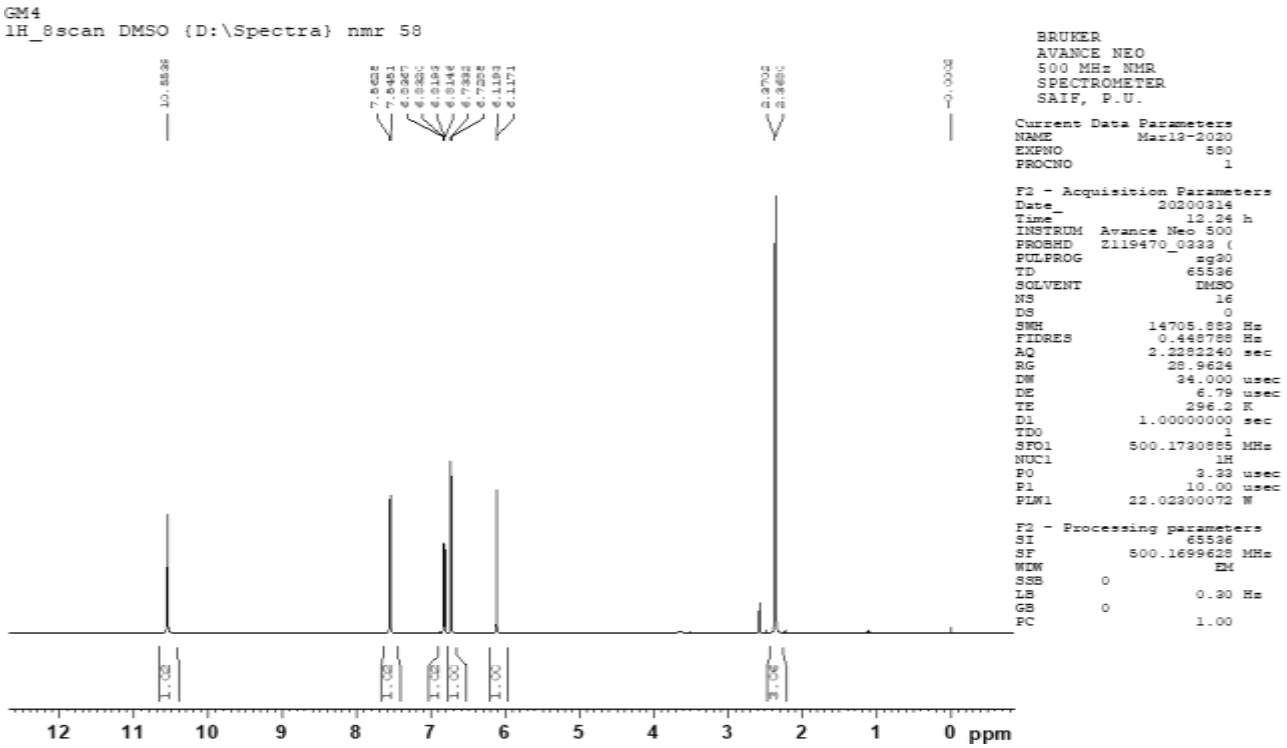


Fig. 12: ^{1}H- spectrum 7-hydroxy-4-methyl-3-acetyl-2H-chromen-2-one (HMAC)

Table 5
Mass spectral data (ESI) m/z of substituted derivatives of 3-acetyl 2-*H*-chromene-2-one

Coumarins	Molecular Mass m/e	Mass fragmentation $[M]^{+1}$
MAC	218.20	219
DAC	259.30	260
HAC	204.17	205
HMAC	218.20	2019

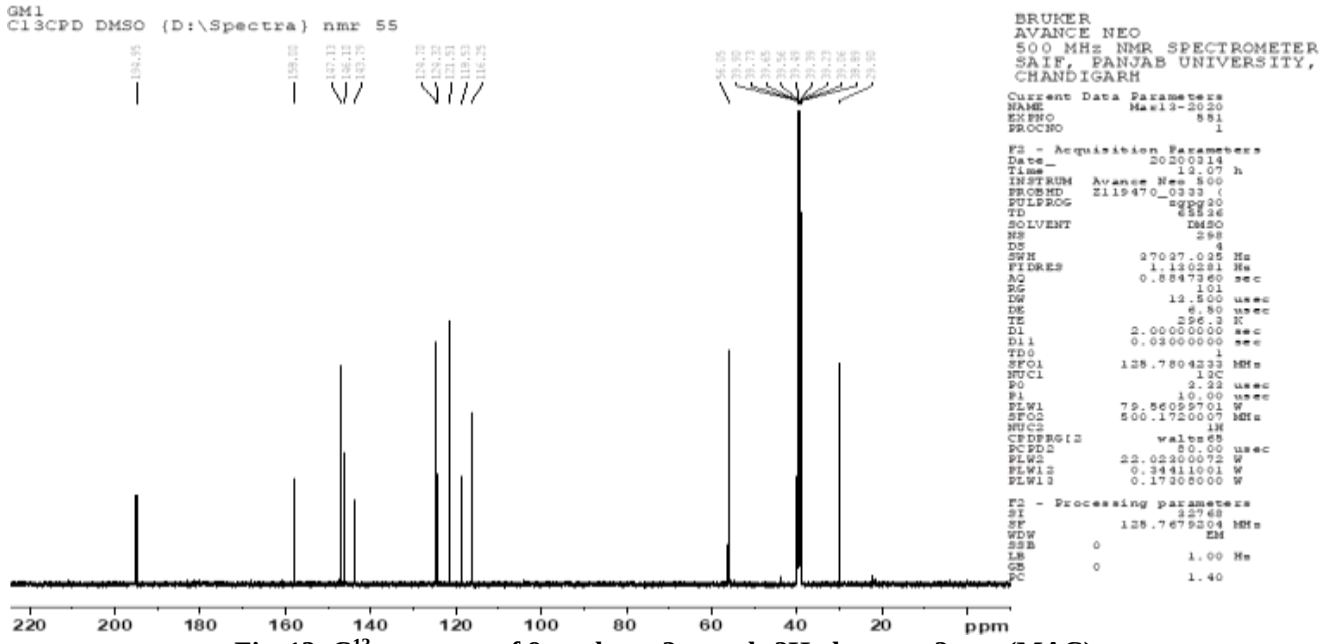


Fig. 13: C^{13} spectrum of 8-methoxy-3-acetyl--2H-chromen-2-one (MAC)

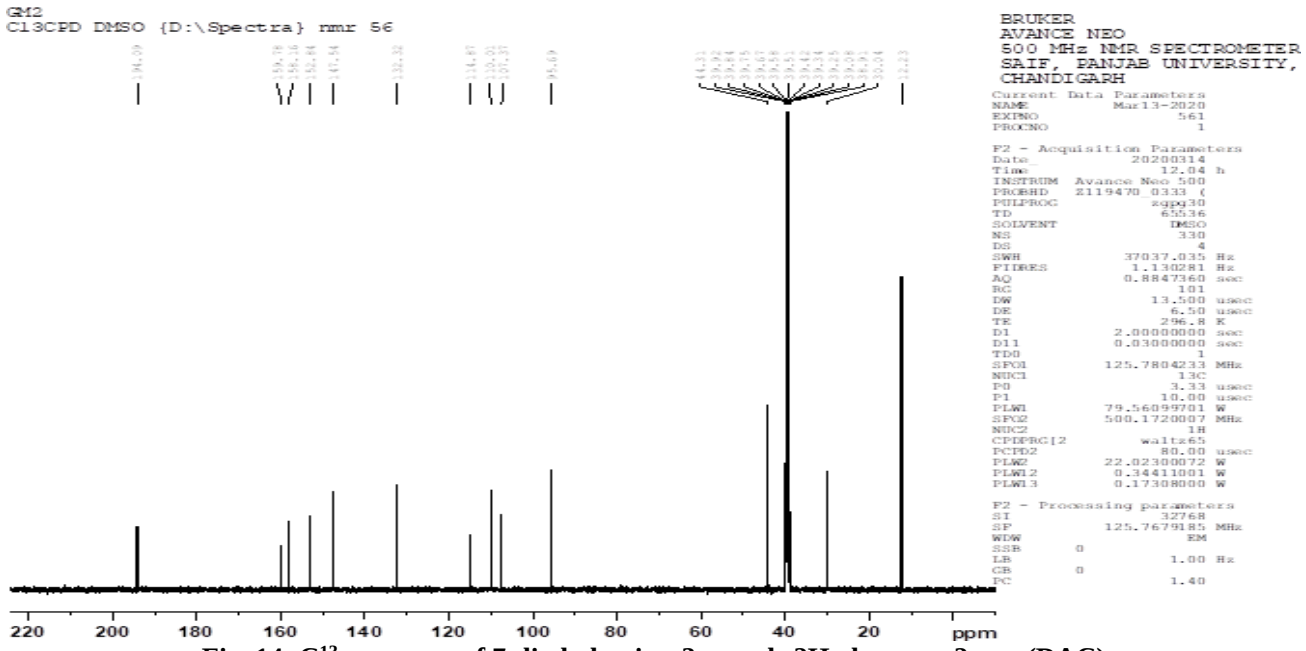


Fig. 14: C^{13} spectrum of 7-diethylamino-3-acetyl--2H-chromen-2-one (DAC)

Table 6
Electronic spectral data of subtd. 3-acetyl 2-H-chromene-2-one chromophores

Entry	Maxi. absorbs at λ nm conc.10^{-4}M in DMSO	Auxo chromophores Substituents	Important Transitions
MAC	228, 269,317	>C=O,O=CCH_3 and >C=C<	$\pi \rightarrow \pi^*$, n $\rightarrow \pi^*$ $\pi \rightarrow \pi^*$.
DAC	340, 436,581	ArN<, >C=O, O=CCH_3, >C=C<	$\pi \rightarrow \pi^*$, n $\rightarrow \pi^*$, $\pi \rightarrow \pi^*$.
HAC	215,315	Ar-OH, >C=O,, >C=C<	n $\rightarrow \sigma^*$, $\pi \rightarrow \pi^*$, n $\rightarrow \pi^*$
HMAC	322, 368, 397.	Ar- OH, >C=O,>C=C<	n $\rightarrow \sigma^*$, $\pi \rightarrow \pi^*$, n $\rightarrow \pi^*$

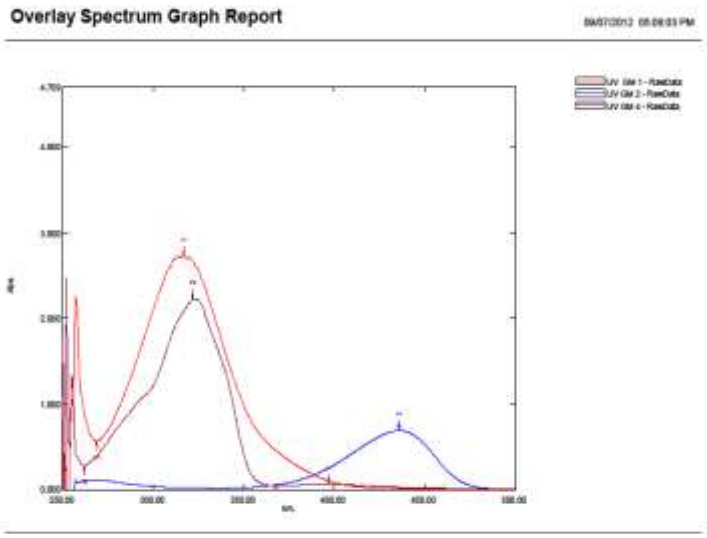


Fig. 15: Overlay Electronic spectrum spectrum of 8-methoxy-3-acetyl--2H-chromen-2-one (MAC), 7-diethylamino-3-acetyl--2H-chromen-2-one (DAC), 7-hydroxy-4-methyl-3-acetyl-2H-chromen-2-one (HMAC)

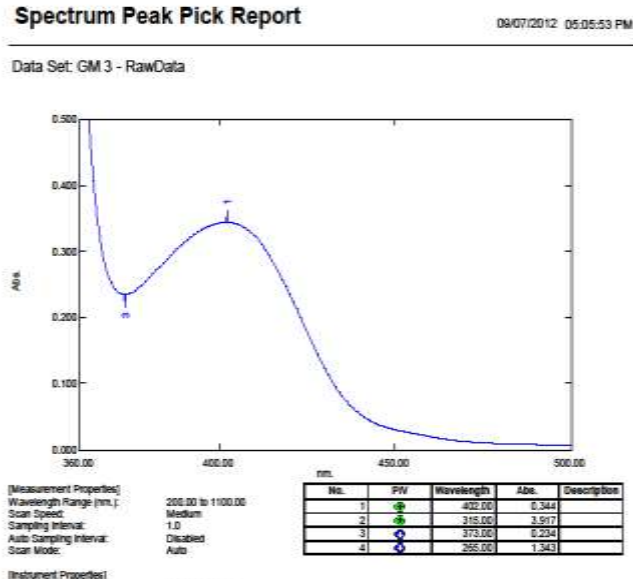


Fig. 16: Electronic spectrum of 7-hydroxy-3-acetyl--2H-chromen-2-one (HAC)

Electronic Spectra: The attachment of substituent group on basic chromophores changes the position and intensity of absorption band of the chromophores. The substituents increases the intensities of absorption due to auxochromses >C=O, O=CCH_3 and >C=C<; -OH and amino groups profoundly show important transition in electronic spectrums in fig. 15 and fig. 16.

Biological Screening: Mono microbial sensitivity disc was used to evaluate the *in vitro* susceptibility of antimicrobial agents of rapidly growing bacteria.[23]

The Potato dextrose agar pH 7.3 was poured into plates kept on a levelled surface. The depth of medium should be approx. 4mm. After the medium was solidified, dry the plate for 30 min. in incubator $+35^0$ to $+ 37^0$ to remove excess moisture from surface.

Use pure culture for gram staining before preparing an inculum. Selected 4-5 similar colonies were transferred into a tube containing 5 ml of Trypticase soya broth. Colonies were directly suspended into a small volume of saline.

Dip a sterile cotton swab into diluted culture inoculum and rotate inside wall of tube. A sterile cotton swab was dipped in diluted culture inoculum. The plates were incubated at temperature $+35^0$ to $+ 37^0$ for 16 to 18 hours.

Measure the diameter of zone of inhibition at last of incubation period. The size of inhibition zone was considered. The zone size interpretative chart as per literature is represented in table no. 7.

Table 7
Zones of Inhibition of Growth of Microorganism

Compounds	GRAM -VE BACTERIA		GRAM + VE BACTERIA	
	Escherichia coli **(mm)**	***Salmonella typhi*** **(mm)**	***Staphylococcus aures*** **(mm)**	***Bacillus substillis*** **(mm)**
MAC	14	20	11	----
DAC	15	25	10	10
HAC	20	15	11	10
HMAC	15	10	----	----
Reference Antibiotic	30 mm (Ofloxacin)	30 mm (Ofloxacin)	15 mm (Azithromycin)	20 mm (Azithromycin)

Conclusion

3-acetyl derivatives of coumarin were synthesized using convenient method without solvent and characterized by different techniques. These coumarins consist of auxochromophore having strong absorption and indicating the solvatochromics. The biological screening of coumarin shows potent against bacterial colonies of *Escherichia coli Salmonella typhi*, *Staphylococcus aures* and *Bacillus substillis.*

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(Received 28th March 2020, accepted 31st May 2020)