International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.15, pp 126-134, 2017

Synthesis of New coumarin substituted chromone with reference to their Anti-bacterial efficacy and fluorescence properties

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Abstract : The novel series of coumarin substituted chromone are important building block in organic chemistry and used as valuable synthetic intermediates in the preparation of new biological relevant heterocyclic system have been synthesized by using effective and efficient synthetic pathway. The synthesized derivatives have been characterized by FTIR, ^{1}HNMR and UV spectroscopy for their structural elucidation. These coumarin substituted were evaluated for their antibacterial activity against different pathogenic bacteria such as *salmonella typhi, Protenus vulgaris, Staphylococcus aureus, klebsiella pnemoniae, E.schrichia, shigella flexneri*. Fluorescent properties of these compounds were also studied.
Keyword : Coumarin substituted chromone, Antibacterial efficacy, Fluorescence properties

1. Introduction

The chromone ring system or 1-4 benzopyrone is a derivative of benzopyrane substituted keto group on the pyran ring, these derivatives are isomeric with respect to coumarin. The word chromone is derived from the Greek word chroma, which indicates that many chromone derivatives exhibit a broad variation of color spectrum.

Chromone constitute a major class of naturally occurring compounds which are biologically active[1]. Therefore, in recent time the chemistry of these building blocks seems to be quite exploring. The general structure of chromone heterocyclic system is shown in Fig. 1 Heterocyclic compounds plays an important role in the design and discovery of new physiological active compounds.[2] Chromone are widely spread in nature and possess multiple biological properties like antibacterial[3-4], antifungal[5-10], anticancer[11-12] and antioxidant[13-15].

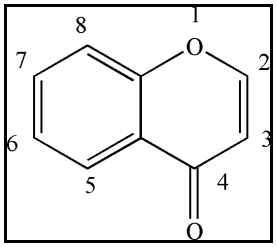


Fig. 1: The general structure and numbering of chromone

Several chromone derivatives have also been reported to act kinase inhibitors to bind to benzodiazepine receptors and as efficient agent in the treatment of cystic fibrosis[16]. Current medical treatment with chromone derivatives can be exemplified by sodium chromoglycate used as a mast cell stabilizer in allergic asthma [17].

Coumarin have been used as an additive in food, cosmetic as well as in the preparation of optical brighteners, laser dyes, and fluorescent compounds [18] .There are many literature review on various synthetic methods that can be used for the preparation of coumarin substituted chromone compounds[19,20]. Coumarin can be prepared by a Perkin reaction between salicylic aldehyde and acetic anhydride. The Pechmann condensation provides another way of synthesis of coumarin and its derivatives. Daniel *et al* [21] used bulk and silica supported wells Dawson acid ($H_2P_2W_{18}O_{62}.24H_2O$) as reusable, heterogeneous catalyst to obtain substituted chromones for cyclisation of 1-(2-hydroxyphenyl)-3-aryl-1,3-propanediones[22]. They are important building blocks in organic chemistry and are used as valuable synthetic intermediates in the preparation of new biologically relevant heterocyclic systems [23-26].

In present research, we have developed new ecofriendly synthetic pathway for the synthesis of coumarin substituted chromone which can be utilized for evaluation of their biological and fluorescence applications.

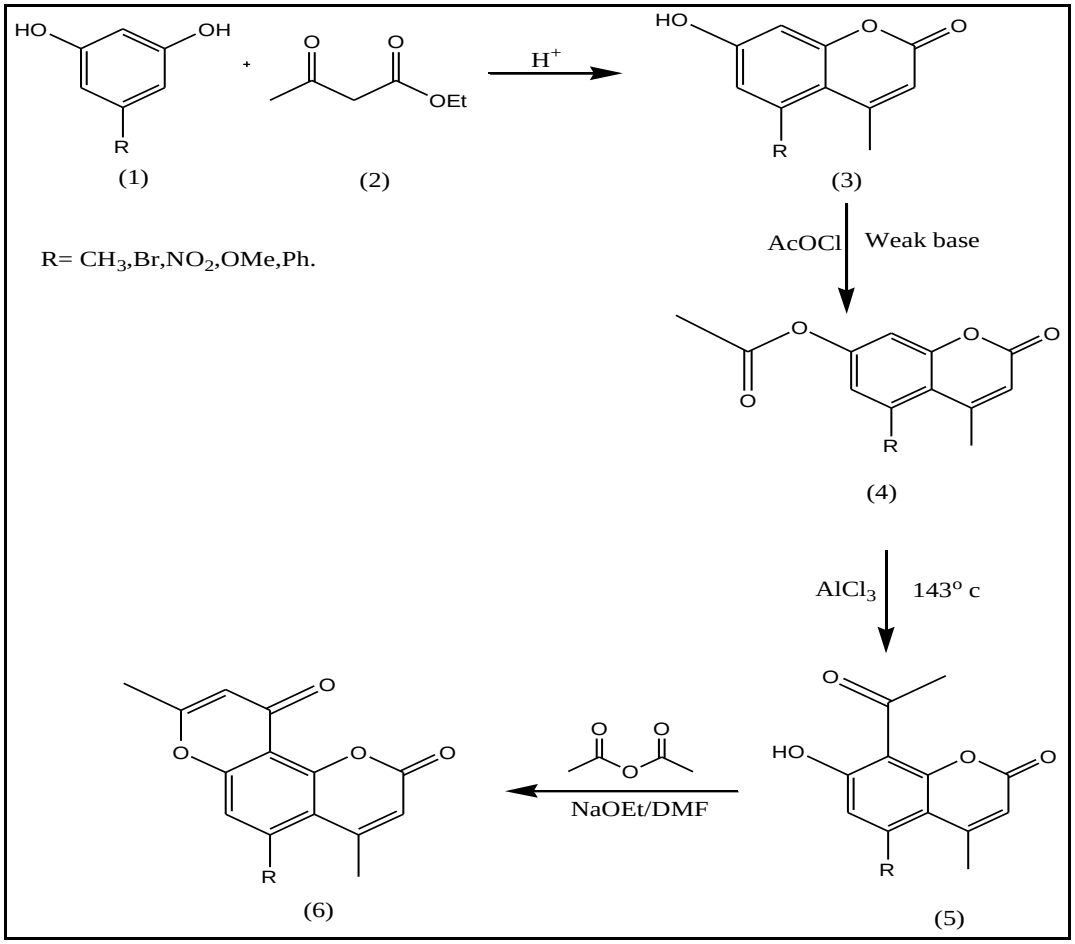


Fig. 2: Synthetic for the preparation of coumarin substituted chromone

2. Experimental

2.1 Raw Materials and Chemicals

Resorcinol (98%), acetic anhydride (99%) was procured from Sigma Aldrich and used as received. Ethyl acetoacetate (98%), TLC plates (silica gel 60 F 254) and Acetyl chloride (98%) was received form Merck chemicals. Acetone (98%) and H_2SO_4 (98%) was provided by Fisher scientific chemicals. Dimethylforamide (S.D. fine chemicals) , NaOH (Ranchem), $AlCl_3$ (Loba chemie).

2.2 Synthesis of 7-Hydroxy -4 -Methyl- 2-H-Chromen-2-One by Pechmann Condensation

A 100 ml Erlenmeyer conical flask equipped over magnetic stirrer was charge with resorcinol (5 g/mol) containing ethyl acetoacetate as solvent (5.9g/mol). The reaction mass was cooled down up to 10^0C and concentrated H_2SO_4 (10ml) was added slowly to the reaction mass with constant stirring. Afterword, the reaction was continuing over the period of 10 hr in anhydrous condition. The progress of reaction was monitored by TLC. On completion of reaction, the reaction mass was poured over crushed ice resulted in the formation of white amorphous solid. The formed solid was filtered, washed with water, dried and crystallized from ethanol. The yield of product was found to be 92%.Melting point-180 ^{0}C.

2.3 Synthesis of 4-Methyl-2-Oxo-2h-Chromon-7-Yl Acetate By Acetylation

A 100 ml Erlenmeyer conical flask equipped over magnetic stirrer was charge with 7-hydroxy -4 -methyl- 2-H-Chromen-2-one (1g/mol), NaOH (1g/mol) and acetyl chloride (1.5 g/mol). The reaction mass stirred gently over the time of 2hr, the progress of reaction was monitored by TLC. On completion of reaction, the reaction mass was poured over crushed ice resulted in the formation of brown amorphous solid. The formed solid was filtered, washed with water, dried and crystallized from ethanol. The yield of product was found to be 88%. Melting point 200 ^{0}C.

2.4 Synthesis of 8-Acetyl-7-Hydroxy-4-Methyl-2h-Chromen-2-One By Fries Rearrangement

100 ml round bottom flask equipped with reflux condenser thermocouple charge with 4-methyl-2-oxo-2H-chromon-7-yl acetate(1g) **,**in solvent (1g) $AlCl_3$, reflux for 2 hour in oil bath and maintained the temperature 135 to 145^{0}C then add 1% HCl for 12 hour. The progress of reaction was monitored by TLC at room temperature. on completion of reaction, the reaction mass was poured over crushed ice resulted in the formation of black crystalline solid. The formed solid dried and crystallized from ethanol. The yield of product was found to be 80%. Melting point-220 ^{0}C.

2.5 Synthesis of (4, 8-Dimethyl–2h,10h-Pyranol(2-3-F)Chromene-2, 10-Dione)

100 ml Erlenmeyer conical flask equipped place over magnetic stirrer, and charge with 8-acetyl-7-hydroxy-4-methyl-2H-chromen-2-one (1g) in NaOEt (1g), acetyl chloride (1.5 ml), dry acetone (15 ml) as solvent also add 40% 9 ml KOH in reaction mixture, reaction mixture gently starting stirring for 8 Hour and The progress of reaction was monitored by TLC.On completion of reaction, the reaction mass was poured over crushed ice resulted in the formation of brown amorphous solid. dried and crystallized from ethanol. The yield of product was found to be 78%. Melting point –260^{0}C;

2.6 Analytics

FT-IR analysis

The FT-IR analysis was conducted on Shimadzo (8400 s, Japan) instrument using the ATR technique and the spectrum was obtained in the wavelength range of 4000–600 cm-1.

Thin layer chromatography (TLC)

Merck silica gel 60 F254. Detection under UV light at 254 nm and 366 nm without dipping Reagent

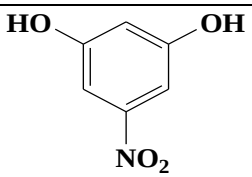
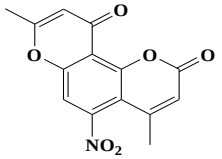
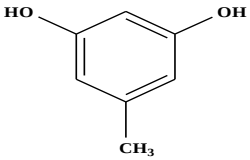
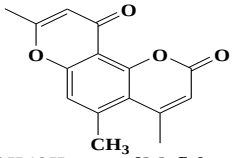
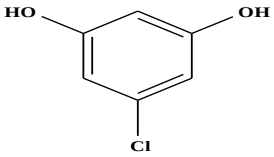
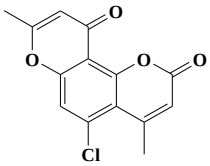
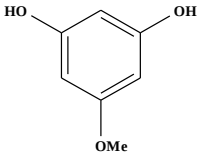
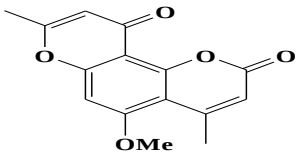
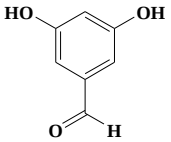
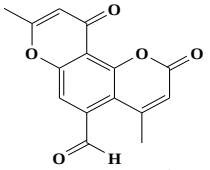
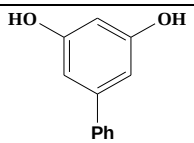
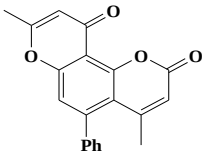
^{1}H-NMR-Spectroscopy

BRUKER AVANCE II 400 The spectra were calibrated according to the solvent signals: 7.26 ppm for CDCl3, 2.50 ppm for DMSO-*d6*.354 Peak characterization: s = singlet, d = doublet, t =triplet, dd=double of doublets, td = triplet of doublets, dt = doublet of triplets, q = quartet, m=multiplet

UV-VIS Analysis

Instrument Cary 60 ,instrument version 2.00,X mode Nanometer, Y mode Abs ,UV-Vis scan rate (nm/min) 24000.00,Ave.Time (sec) 0.0125,Beam Mode-Dual Beam

Table 1 : Structures and physical properties of the synthesized compounds

Sr. No.	Entry	Product	Molecular weight [a]	Yield[b]
1.	 5-nitrobenzene-1,3-diol	 4,8-dimethyl-5-nitro-2*H*,10*H*-pyrano[2,3-*f*]chromene-2,10-dione	287.22	65%
2.	 5-methylbenzene-1,3-diol	 4,5,8-trimethyl-2*H*,10*H*-pyrano[2,3-*f*]chromene-2,10-dione	256.25	70%
3.	 5-chlorobenzene-1,3-diol	 5-chloro-4,8-dimethyl-2*H*,10*H*-pyrano[2,3-*f*]chromene-2,10-dione	276.67	50%
4.	 5-methoxybenzene-1,3-diol	 5-methoxy-4,8-dimethyl-2*H*,10*H*-pyrano[2,3-*f*]chromene-2,10-dione	272.25	65%
5.	 3,5-dihydroxybenzaldehyde	 4,8-dimethyl-2,10-dioxo-2*H*,10*H*-pyrano[2,3-*f*]chromene-5-carbaldehyde	270.23	60%
6.	 5-phenylbenzene-1,3-diol	 5-phenyl-4,8-dimethyl-2*H*,10*H*-pyrano[2,3-*f*]chromene-2,10-dione	318.32	55%

a- -molecular weight of isolated product
b- -yield of isolated product

3. Evaluation of Antibacterial Properties

Synthesized coumarin substituted chromone were screened for their antibacterial activities against pathogenic bacteria such as *salmonella typhi, Proteus vulgaris,Staphylococcus aureas, klebsiella pnemoniae, E.schrichia, shigella flexneri* by using agar-agar well diffusion method. The test compound were dissolved in dimethyl sulphoxide at a concentration of 100 ug/ml using ciprofloxin (20ug) us standard drug. All the inoculated plates were incubated at 37^0C and the result were evaluated after 24 hour of incubation.

4. Fluorescence Properties

The fluorescence spectra were recorded on F-7000 FL spectrofluorometer in ethanol at a concentration of 1mg mL^{-1}Calculation of the quantum yield,

Φx = $\Phi s[As/Ax][Rs/Rx][D/Ds]$
Φ = Fluorescence quantum yield
Subscripts x and s denotes test and standard respectively
R = refractive index of the solvent
D = area under the corrected, extrapolated emission spectra [27,28].

5. Result and Discussion

5.1 Synthesis of coumarin derivatives

The result obtained in the present study showed that this information would give rise to design of better molecule with good yields, developed of new synthetic green strategies and efficient is desirable for the synthesis of coumarin substituted chromone.

5.2 Spectral analysis

^{1}H NMR spectral analysis;

δ 7.79 (s, 1H), 7.20 (d, *J* = 27.7 Hz, 2H), 6.36 (s, 1H), 2.42 (s, 3H), 2.30 (s, 3H); shown in (figure; 01)

FT-IR spectral analysis

The general spectral characterization show absorption bond correspond the 1696 cm^{-1} for ν(c=o) stretching of coumarin moiety. It is generally occurring at 1725 cm^{-1} but due to aromatic conjugation it reduces to 1696 cm^{-1}. the most prominent bond due to (–c-o-c) stretch occur at range 1396 -1012 cm^{-1} .it is also observe that weak bond at 857 cm^{-1}due to symmetric stretch of ether the characteristics absorption peak at 1617 cm^{-1} assign to ν (c=c) stretching .

UV spectral analysis

The absorbance versus wavelength was measured by UV-visible spectrometer for new synthesis coumarin substituted chromone. The recorded absorption spectrum is shown in Fig.02. The graph reveals that synthesized compound a wider transparency range extending into entire visible and absorbance takes place in the UV range 325nm to 410nm. And the cut off wavelength (X cm-off) within the range between 340 to 210 nm in Ethyl acetoacetate with λ-max was found to be 285nm.This absorbance maximum is to be assigned to $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$ transition and may be attributed to the excitation in the aromatic ring and C= O group.

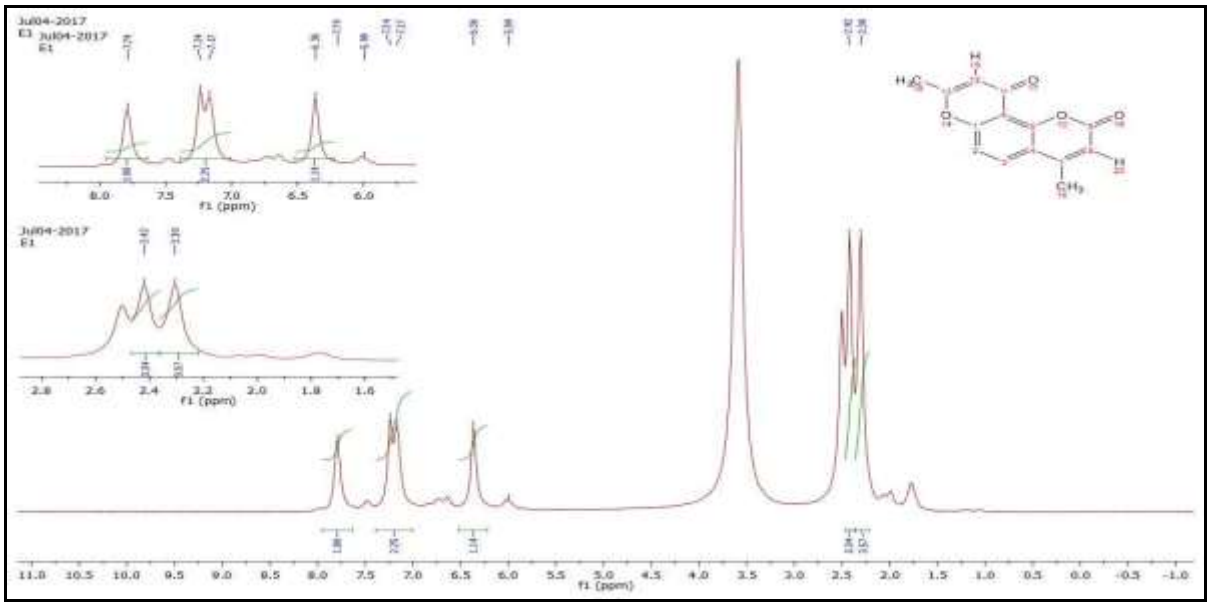


Fig. 1: ^{1}H NMR spectrum of compound

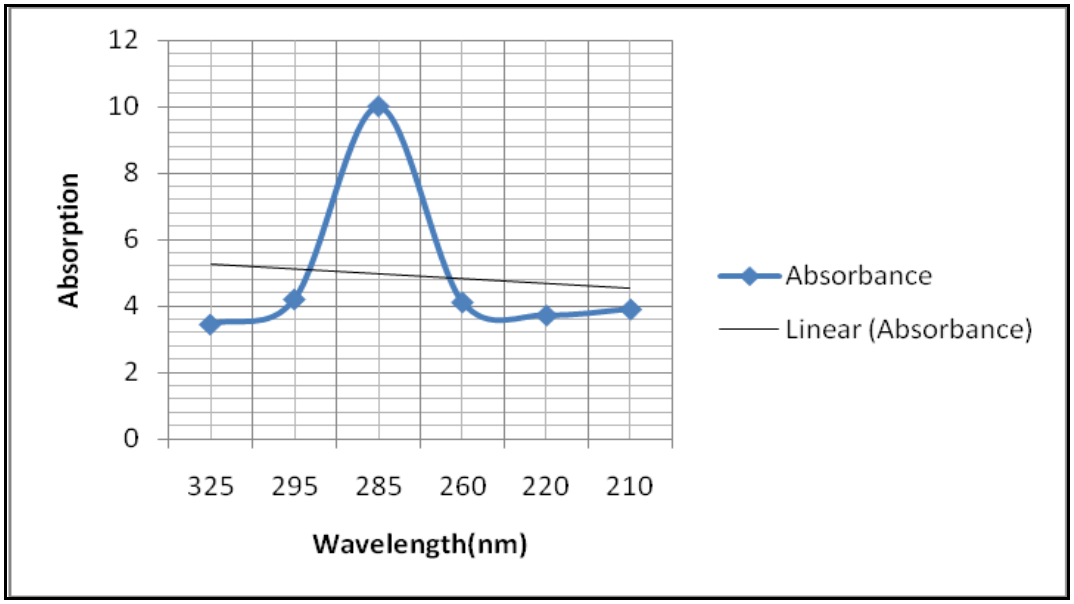


Fig. 2 : UV-Vis spectrum of compound

5.3 Antimicrobial Activity

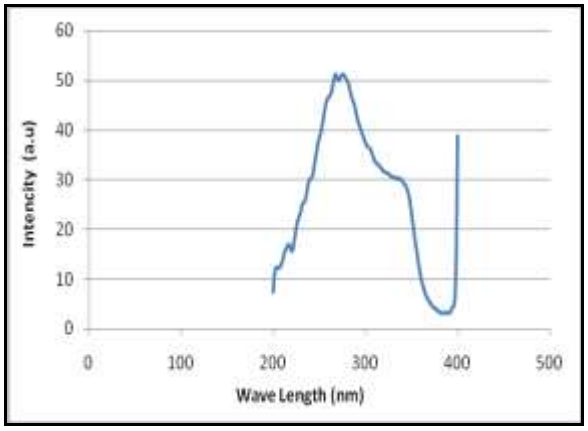
After incubation for 24 hr, samples were analyzed for zone of inhabitation. It was observed that coumarin substituted chromone derivative shows pronoused antibacterial activity in case of gram negative bacteria (*salmonella typhi, Proteus vulgaris, klebsiella pnemoniae, E.schrichia, shigella flexneri*) because it thick, cross link And the gram positive *(Staphylococcus aureus)* less pronoused because of thin cell membrane. Chemically Coumarin substituted chromone (4,8-dimethyl–2H,10H-Pyranol(2-3-f) Chromene-2, 10-dione) are heterocyclic compounds with the benzo-c-pyrone framework. Molecules containing the chromone or benzopyranone ring have a wide range of antibacterial activities.[29] Therefore the vast range of biological effects associated with this scaffold has resulted in the chromone ring system being considered as a privileged structure.[30-31]

Zone of inhibition

Salmonellatyphi-10mm(std-26mm),proteusvulgaris-26mm (std-29mm), Staphylococcus auseus 15 mm (std-31mm), klesiella pnemonide-10mm (std-27mm),E-Coli-21mm (std-28mm), shigella flexnari-11mm (std- 29 mm). [Activity index –std][Zone of inhibition-mm]

5.4 Fluorescent Properties

The studies of fluorescence were performed in ethanol at a concentration of 1.0×10-5 mol/L and the fluorescence quantum yields (Table1) of compounds (1-6) were determined via comparison method. Fluorescence spectra were obtained at their respective maximum excitation wavelength. Coumarin substituted chromone derivatives substituted an electron-donating group were known to exhibit strong fluorescence. The electron donating substituent group 1-6 exhibited longer emission wavelength ranging from 396 to 440 nm, where benzocoumarin part served as strong donor [32-35] to constitute a strong push-pull system to exhibit high fluorescent intensities. The fluorescence spectral data of compound [A] and 1-6 are summarized in fig. 1, 2 and 3,4.



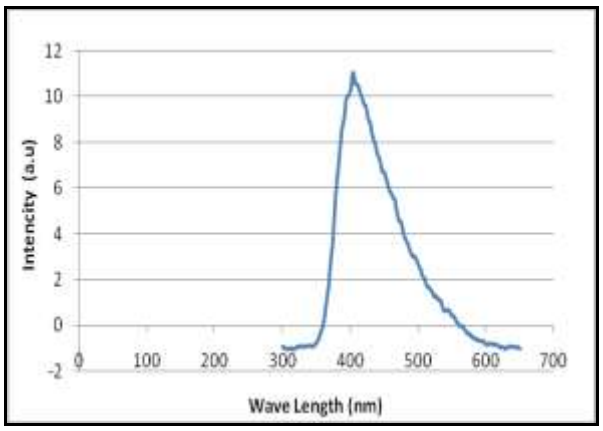
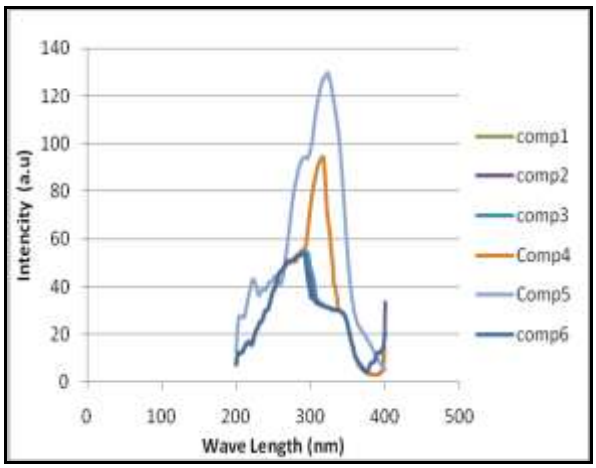


Fig. 1:Excitation spectra of compound [A] **Fig.2:Fluorescence emission of compound [A]**



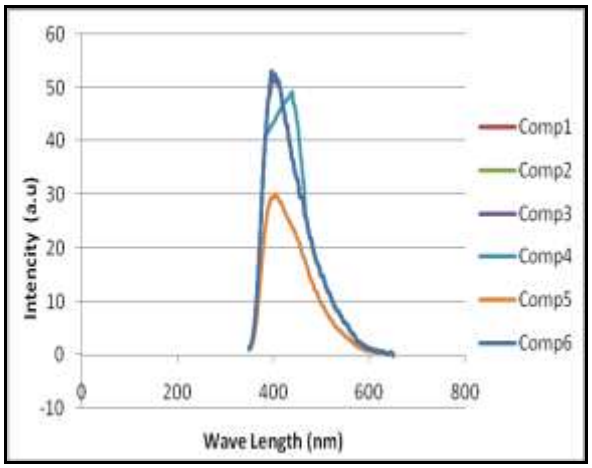


Fig. 3: Excitation spectra of compound 1-6 **Fig.4: Fluorescence emission of compound 1-6**

Table 1: Electronic absorption (UV λ Max), emission (Em λ Max) and Stoke shifts of compounds (1-6)

No.	Excitation (nm)	Emission (nm)	Stoke shifts (nm)
1	330	398	68
2	293	397	104
3	295	397	102
4	294	440	146
5	323	405	82
6	325	396	71

6. Conclusion

In this study, we have successfully synthesized Coumarin substituted chromone and structure were confirmed by spectral data performed by IR, NMR, and UV-visible absorption data reflect the extent of electron delocalization and the ground state electron structure of the materials. Compound so obtained were further investigated for their antibacterial activity which show some significant results in case of Gram- negative *(salmonella typhi, Proteus vulgaris, klebsiella pnemoniae, E-coli, shigella flexneri*) bacteria pronoused compared with Gram-positive *(Staphylococcus aureus)* bacteria. The fluorescence properties of the synthesized compounds were studied in ethanol. The results obtained were interesting that the compounds show fluorescent in ethanol with good quantum yield and show high activity.

Acknowledgement

The authors are thankful to Sophistigated Analytical instrumentation Facility (SAIF), Panjab University, Chandigarh for providing spectral data. We are thankful to Head, Department of Physics, Sant Gadge Baba Amravati University, Amravati, for providing fluorescence data. We are also thankful to Sudhakarrao Naik Pharmacy Institute, Pusad (M.S.) for providing antibacterial activity.

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