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RESEARCH ARTICLE

SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME 4-HYDROXY-3-[3-(SUBSTITUTED PHENYL)- PROP-2-ENOYL]-2H-CHROMEN-2-ONES

Vishal P. Narodia, Milan S. Vadodaria, Govind Vagadiya and *Kartik D. Ladva

Department of Chemistry and Industrial Chemistry, Shree M. & N. Virani Science College, Yogidham, Kalawad Road, Rajkot – 360005, Gujarat State, India

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ABSTRACT

Due to the presence of α,β-unsaturated ketone chalcone derivatives from nature or synthetic provenance display manifold pharmacological activities. The present work deals with the unfolding of the biological potency, if any, of some of the newly synthesized benzalacetophenones. Out of the 20 homologues synthesized the 6 were found active against the bacterial strain and 3 were active against fungal strains. The structures of the compounds were supported by UV, IR, NMR, Mass spectroscopy and elemental analysis.

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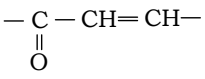
INTRODUCTION

The Chalcones or phenyl styryl ketones areα,β unsaturated ketones, containing the reactive keto-ethylenic group:



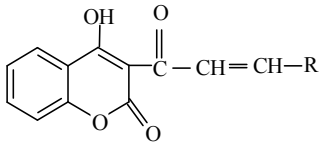
These compounds are also known as benzylideneacetophenones or benzalacetophenones, which are named "Chalkones" by Kostanecki and J.Tambor (Kostanecki and Tambor, 1921). Chalcones are natural products rampantly found in plants. Due to the presence of α,β-unsaturated ketonechalcone derivatives from nature or synthetic provenance displaymanifold pharmacological activitiesbeing antimicrobial (Prasad *et al*., 2006), antitumor (Kumar *et al*., 2003), anticancer (Tatsuzaki *et al*., 2006; Yun *et al*., 2006), radical scavenger (Kim *et al*., 2008), inhibitor of to poisomerase I (Yoon *et al*., 2007), antihepatotoxic activity (Khan *et al*., 2006), antiplasmodial activity (Wu *et al*,. 2006), antibacterial activity (bashkar and Reddy, 2011), antimitotic activity (Achanta *et al*., 2006) etc. Coumarin is a fragrant organic compound known as 1,2-benzopyrone, consisting of fused benzene and α-pyrone rings, present in significant amounts in plants and more than 1300 coumarins were identified from natural sources (Hoult and Paya, 1996). The coumarins also show activity such as antifungal (Sangwan *et al*., 1990), anticoagulant (Stahman *et al*., 1941), antibacterial (Honmantgad *et al*., 1985), insecticidal (Hapworth, 1984), anti HIV (Desai *et al*., 2008), antiallergic (Bhanvesh Naik and Desai, 2006), antiasthmatic (Siddiqui *et al*., 2010), antimalarial (Matloubi Moghaddam *et al*., 2009), antiviral (AntigoniKotali *et al*., 2008), anti-inflammatory (Vinod Kumar Pandey *et al*., 2004), antichemotherapeutics (Noyce *et al*., 1955), antileucemic activity (Hariprasad *et al*., 1998), antihypertensive activity (Joshi, 2001) and cytotoxicactivity (Shah *et al*., 1960). With this varied pharmacological activities possessed by the above moieties the novel 4-hydroxy coumarin derivatives possessing various aromatic components with chalcone group has been synthesized by us.

Corresponding author: Kartik D. Ladva,
Department of Chemistry and Industrial Chemistry, Shree M. & N. Virani Science College, Yogidham, Kalawad Road, Rajkot – 360005, Gujarat State, India.

MATERIALS AND METHODS

The two routes have been applied for the synthesis of title compounds. First route consists of the preparation of styryl

Table 1. Physical data of 4-Hydroxy-3-[3-(substituted phenyl) prop-2-enoyl]-2H-chromen-2-ones [Values in Parenthesis denotes the calculated percentage of composition]



S.No.	R	Molecular Formula	M. wt. gm/mole	M. P. (ºC)	Elemental analysis		
					C	H	N
1.	2.	3.	4.	5.	6.	7.	8.
1	3-Methoxy-4-hydroxy phenyl	$C_{19}H_{14}O_6$	338	185	67.40 (67.45)	4.12 (4.14)	-
2	4-Thiomethyl phenyl	$C_{19}H_{14}O_4S$	338	190	67.48 (67.45)	4.10 (4.14)	-
3	2-Chloro phenyl	$C_{18}H_{11}Cl\ O_4$	326.5	140	69.80 (69.83)	3.40 (3.36)	-
4	2-Nitro phenyl	$C_{18}H_{11}NO_6$	337	140	64.10 (64.09)	3.24 (3.26)	4.15 (4.14)
5	4-Methoxy phenyl	$C_{19}H_{14}O_5$	322	220	70.82 (70.80)	4.35 (4.34)	-
6	4-Chloro phenyl	$C_{18}H_{11}ClO_4$	326.5	180	69.80 (69.83)	3.31 (3.36)	-
7	4-N,N-Dimethyl phenyl	$C_{20}H_{17}NO_4$	335	180	71.70 (71.64)	5.10 (5.07)	4.15 (4.17)
8	3-Nitro phenyl	$C_{18}H_{11}NO_6$	337	180	64.10 (64.09)	3.20 (3.26)	4.10 (4.15)
9	2-Hydroxy phenyl	$C_{18}H_{12}O_5$	308	170	70.15 (70.12)	3.91 (3.89)	-
10	3-Phenoxy phenyl	$C_{24}H_{16}O_5$	384	190	75.05 (75.00)	4.20 (4.16)	-
11	3-Bromo phenyl	$C_{18}H_{11}BrO_4$	371	200	58.29 (58.22)	3.00 (2.96)	-
12	2,5 – Dichloro phenyl	$C_{18}H_{10}Cl_2O_4$	360	160	60.07 (60.00)	2.80 (2.77)	-
13	2,4 – Dichloro phenyl	$C_{18}H_{10}Cl_2O_4$	360	170	60.01 (60.00)	2.75 (2.77)	-
14	3,4 – Dimethoxy phenyl	$C_{20}H_{16}O_6$	352	140	68.15 (68.18)	4.50 (4.54)	-
15	4- Fluorophenyl	$C_{18}H_{11}FO_4$	310	130	69395 (69.90)	3.40 (3.55)	-
16	Phenyl	$C_{18}H_{12}O_4$	292	170	73.99 (73.97)	4.13 (4.10)	-
17	3-Chloro phenyl	$C_{18}H_{11}ClO_4$	326.5	150	66.20 (66.15)	3.30 (3.36)	-
18	3-Indolyle	$C_{20}H_{13}NO_4$	331	140	72.45 (72.50)	3.90 (3.92)	4.20 (4.22)
19	Phenethyl	$C_{20}H_{16}O_4$	320	190	75.05 (75.00)	5.02 (5.00)	-
20	3,4,5-Trimethoxy phenyl	$C_{21}H_{18}O_7$	382	170	65.91 (65.96)	4.70 (4.71)	-

Table 2.

Code	$\lambda_1(\varepsilon)$	$\lambda_2(\varepsilon)$	$\lambda_3(\varepsilon)$	$\lambda_4(\varepsilon)$	$\lambda_5(\varepsilon)$
VCH-1(N)	-	253 (0.332)	-	394 (0.154)	-
VCH-1(A)	226 (0.280)	259 (0.239)	334 (0.154)	-	424 (0.455)
VCH-1(B)	235 (0.640)	271 (0.519)	-	-	406 (0.481)
VCH-2(N)	-	253 (0.130)	-	376 (0.113)	-
VCH-2(A)	226 (0.235)	262 (0.161)	-	-	412 (0.145)
VCH-2(B)	235 (0.555)	271 (0.412)	-	379 (0.255)	-
VCH-7(N)	-	256 (0.063)	-	-	460 (0.047) 481 (0.048)
VCH-7(A)	226 (0.139)	262 (0.016)	-	-	508 (0.074)
VCH-7(B)	235 (0.475)	271 (0.365)	338 (0.238)	-	-
VCH-8(N)	232 (0.710)	268 (0.710)	328 (0.660) 346 (0.666)	-	-
VCH-8(A)	232 (0.826)	256 (0.703)	358 (0.980)	-	-
VCH-8(B)	238 (1.429)	274 (1.809)	349 (0.745)	-	-

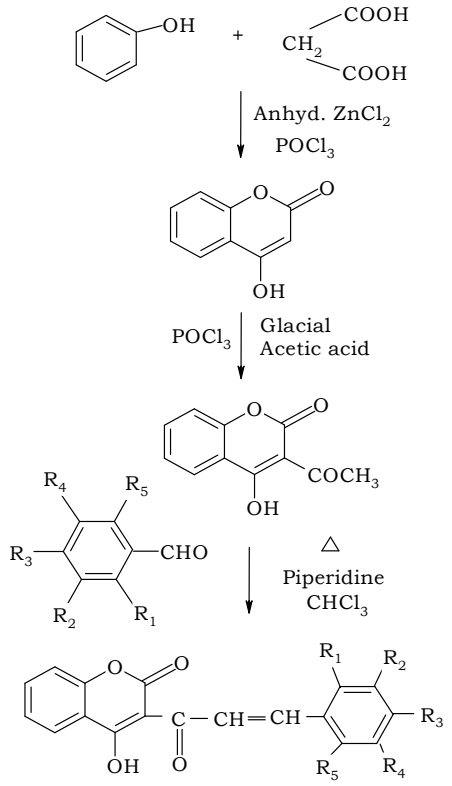
❖ Dark value indicate λ_{max} of compound in different media

derivatives from 2-hydroxyacetophenone and 4-substituted benzaldehydes. The resulting styryl intermediates were further cyclized in presence of hydrogen peroxide and sodium hydroxide. In another route, the 4-hydroxycoumarins were directly condensed with cinnamoyl chloride (Shah *et al*., 1984). When various aldehydes were condensed with malonic acid in presence of pyridine, substituted cinnamic acid derivatives were obtained, which were further converted into respective acid chlorides. The 4-hydroxycoumarin on further treatment with acid chloride resulted in formation of 3-styryl-4-hydroxycoumarins.

These methods established to arrive at the target compounds are rather multistep and involves the use of expensive aldehydes for conversion into cinnamic acids. The yields reported for these compounds are ranging between 40–60 percent (Dholakia *et al*., 1968). We have found that alternative synthetic route adopted by us has many advantage because

(1) The route is useful for a single step preparation for various 4-hydroxy coumarin substituted at benzenoid part.
(2) The use of aldehyde in the first steps resulting into loss of yield can be eliminated.
(3) The respective of third position of coumarin system greatly facilitates direct formation of 3-acetyl-4-hydroxy coumarins avoiding any possibility of acetylation of hydroxy group at C_4 position.
(4) As use of aldehydes in last step, increase the overall yield and purity.
(5) The use of chloroform as a solvent is very convenient for this method. In very few instance, chloroform is reported as reaction medium. It seems to be very convenient laboratory method (Shah *et al*., 1960).

Reaction Scheme



Where R_1=H, Cl.
R_2 =H , OCH3.
R_3 =H, OH, SCH3, OCH3, Cl, N (CH3)2, F.
R_4=H, NO2, OC6H5, Br, OCH3, Cl.
R_5 =H, Cl, NO2, OH.

Experimental

Preparation of 4-hydroxycoumarin

It was prepared according to method of Shah and coworkers[27, 28]. Phenol (8.78 ml, 0,1 mole) and malonic acid (10.8 gm, 0.1 mole) were added to a mixture of phosphorous oxychloride (40 ml) and anhydrous zinc chloride (30 gm) which was preheated to 60-70 ºC and reaction was heated on a water bath at 70 ºC for 6 hrs. It was then cooled to room temperature and decomposed with ice and water to afford solid, which was filtered and washed with water. It was then treated with 10 % sodium carbonate solution and filtered. The filtrate was slowly acidified with diluted hydrochloric acid. At the neutral point, the precipitates obtained were washed with water and dried. The product was recrystallized from ethanol as brown colour product Yield = 6.5 gm; m. p. = 210 – 212 ºC (Reported (Shah *et al*., 1984; Dholakia *et al*., 1968) m. p. 209-210 ºC).

Preparation of 3-acetyl-4-hydroxycoumarin (Dholakia *et al*., 1968)

It was prepared according to method of Trivedi and coworkers (Karangh, 1963). 4-Hydroxy coumarin (2.75 gm, 0.017 mole) was mixed with glacial acetic acid (15 ml). Phosphorous oxychloride (12 ml) was added slowly to the mixture and it was heated on water bath for 30-35 min., than cooled and poured into crushed ice. A brownish solid obtained. Recrystallized from alcohol, yield 2.1 gm, M.P. 120 ºC, (Reported m. p. 120ºC[29]).

Preparation of 4-hydroxy-3-[3-(substituted phenyl) prop-2-enoyl]-2H-chromen-2-one

In a 100 ml round bottom flask 3-acetyl-4-hydroxy coumarin (0.031 mol) and un/substituted aromatic aldehyde (0.031 mol) were dissolved in 30 ml of chloroform. The catalytic amount of piperidine (0.02 ml) was added and the reaction mixture was refluxed for one and half hour on a water bath. It was then allowed to cool at room temperature. As chloroform was evaporated, solid residue was obtained; it was washed with methanol and recrystallized from ethanol. The physical data of the compounds synthesized by this method are given in Table 1.

Spectral discussion

The constitutions of newly synthesized compounds were supported by UV, IR, NMR and Mass spectra study. The details are as under.

Ultra violet spectral study

The UV spectra of newly synthesized compounds were taken in ELICO SL 159 UV-VIS spectrophotometer. The UV spectra

were taken in rectified spirit are neutral, and also in acidic and alkaline medium. The bathochromic as well as hypsochromic shifts are recorded. The UV spectral trend and changes in electronic transfer band (ET bands) are mentioned in Table. It is observed that in most of the cases λ_{max} values were observed at 226, 232, 235, 256, 268, 274, 358, 376, 394, 424, nm. The other ET bands have appeared at 226,253, 271,334, 349, 379, 406, 481 nm etc. As UV spectra of compounds are taken in neutral, acidic and alkaline medium, characteristic blue and red shift is recorded and also changes in ET bands. The values of λ_{max} and other ET bands are given in respective table.

Infra-Red (IR) Spectral study

The infrared spectra were recorded on SHIMADZU FTIR-8400 spectrophotometer by KBr pellet method. The IR (KBr) spectra of all (un) substituted styryl ketone compounds showed broad band at 3105-3450 cm^{-1} due to hydroxy stretching frequency. The aromatic stretching frequencies were also observed at range of 2921-2840 cm^{-1}. Most of styryl ketones gave carbonyl stretching vibration in the region of 1872-1712 cm^{-1}. The C=C stretching band were observed in region of 1646-1600 cm^{-1}, while due to ether linkage (C-O-C) two bands appeared at 1260cm^{-1} (symmetric) and 1090 cm^{-1} (asymmetric) as stretching bands. The aromatic out of plane bending was observed at 873-824 cm^{-1}. It was further observed that the trans isomers which possess an element of symmetry (i.e. a center of symmetry) does not show stretching frequency of the double bond in present study.

Nuclear Magnetic Resonance (^{1}H NMR) spectral study

The ^{1}H NMR spectra of newly synthesized compounds were taken in BRUKER AC 300 MHz FT NMR spectrometer using $CDCl_3$ +DMSO-d_6 as solvents and TMS as internal standard. In all the derivatives, vinyl protons (CH=CH) were observed at 8.2-8.5 δ as a doublet with coupling constant of 14.89 Hz which proves that the vinyl protons are trans to each other. The aromatic protons are observed in the range of 6.9-8.33 δ as a multiplet.

Mass spectral study

Mass spectra were recorded on JEOL SX-120/DX-6000 spectrometer. All the newly synthesized compounds gave typical molecular ion peak according to their molecular weight. In addition they also showed base peak.

Characterization

UV spectral study of 4-Hydroxy-[-3-(substituted phenyl) prop-2-enoyl]-2H chromen-2-one

In the following Table 2:

(1) The λ_{max} and other λ values shown below in (1) Neutral (N), (2) Acidic (A), (3) Alkaline (B) medium.
(2) All spectra are taken in ethanol.
(3) The λ values are shown in nm and Extinction Coefficient (ε) values are shown in bracket.

4-Hydroxy-3-[3-(3-methoxy-4-hydroxyphenyl)prop-2-enoyl]-2H-chromen-2-one (Compound-1)

IR (KBr)cm^{-1}

3440.8 (OH); 3055.0 (C-H asym); 2920.0 (C-H sym); 1728.1 (C=O); 1612.4 (C=C); 1288.4 (C-O-C sym); 1091.6 (C-O-C asym); 829.3 (C-H, o.o.p.).

^{1}H NMR δ :7.94 (S, 3H, OCH_3); 6.89 (d, 1H, CH= CH); 7.27,7.36 (m, 3H, Ar-H); 7.66 (d, 1H, Ar-H); 7.72 (d, 1H, Ar-H); 8.03 (d, 1H, Ar-H); 8.05 (t, 1H, Ar-H); 8.17 (d, 1H, CH= CH).

Mass (FAB) :M.Wt = 338 gm/ mol ; [m/e (%)]; (M+1) **339 (58.33)**; 323 (8.4); 307 (32.49); 289 (16.66); 189 (9.99); 165 (6.66); **154 (100)**; 136 (68.33), 120 (10.83); 107 (19.99).

4-Hydroxy-3-[3-(4-thiomethylphenyl) prop-2-enoyl]-2H-chromen-2-one (Compound-2)

IR (KBr)cm^{-1}

3436.9 (OH); 3070.5 (C-H asym); 2927.7 (C-H sym); 1720.4 (C=O); 1626.0 (C=C); 1288.0 (C-O-C sym); 1076.1 (C-O-C asym); 860.2 (C-H, o.o.p.).

^{1}H NMR (300 MHz $CDCl_3$ + DMSO-d_6)

δ : 253(s, 3H, SCH_3); 7.32 (M, 3H, Ar-H); 7.65 (d, 2H, Ar-H), 7.73 (t, 1H, Ar-H), 8.07 (m, 2H, Ar-H); 8.33 (d, 1H, CH=CH) (J= 14.89) (trans isomer).

Mass (FAB)

M.Wt = 338 gm/ mol; [m/e (%)]; (M+1) **339 (100)**; 307 (14.16); 289 (9.16); 189 (16.66); 177 (5.83); 154 (28.33); 149 (10.83); 136 (15.83); 107 (8.33).

Antimicrobial Profile of 4-Hydroxy-3-[3-(substituted phenyl) prop-2-enoyl]-2H-chromen-2-ones

The results obtained by standard drug fluconazole in this case are compared in accordance with the protocols of antibacterial/ antifungal activity as mentioned below.

Antibacterial activity (Kawase *et al*., 2001; Chavda *et al*., 2002; Synthesis and biological, 2003)

The purified products were screened for their antibacterial activity. The nutrient agar medium was prepared by the usual method was inoculated aseptically with 0.5 ml for 24 hours old subcultures of staph. Aureus 209p and E.Coli ESS 2231 in separate conical flask at 40-500 C and mixed well by gentle shaking. About 25 ml of the medium were poured and evenly spread in petridish (13 cm in diameter) and 10 mm bore in agar medium and filled with 0.05 ml solution of sample in 10 % DMSO in methanol. The plates were incubated at 370 for 24 hours and he control was also maintained with 0.05 ml of 10% DMSO in methanol in similar manner. The zones of inhibition of the bacterial growth were measured.

Possible mass fragmentation of the compound -2

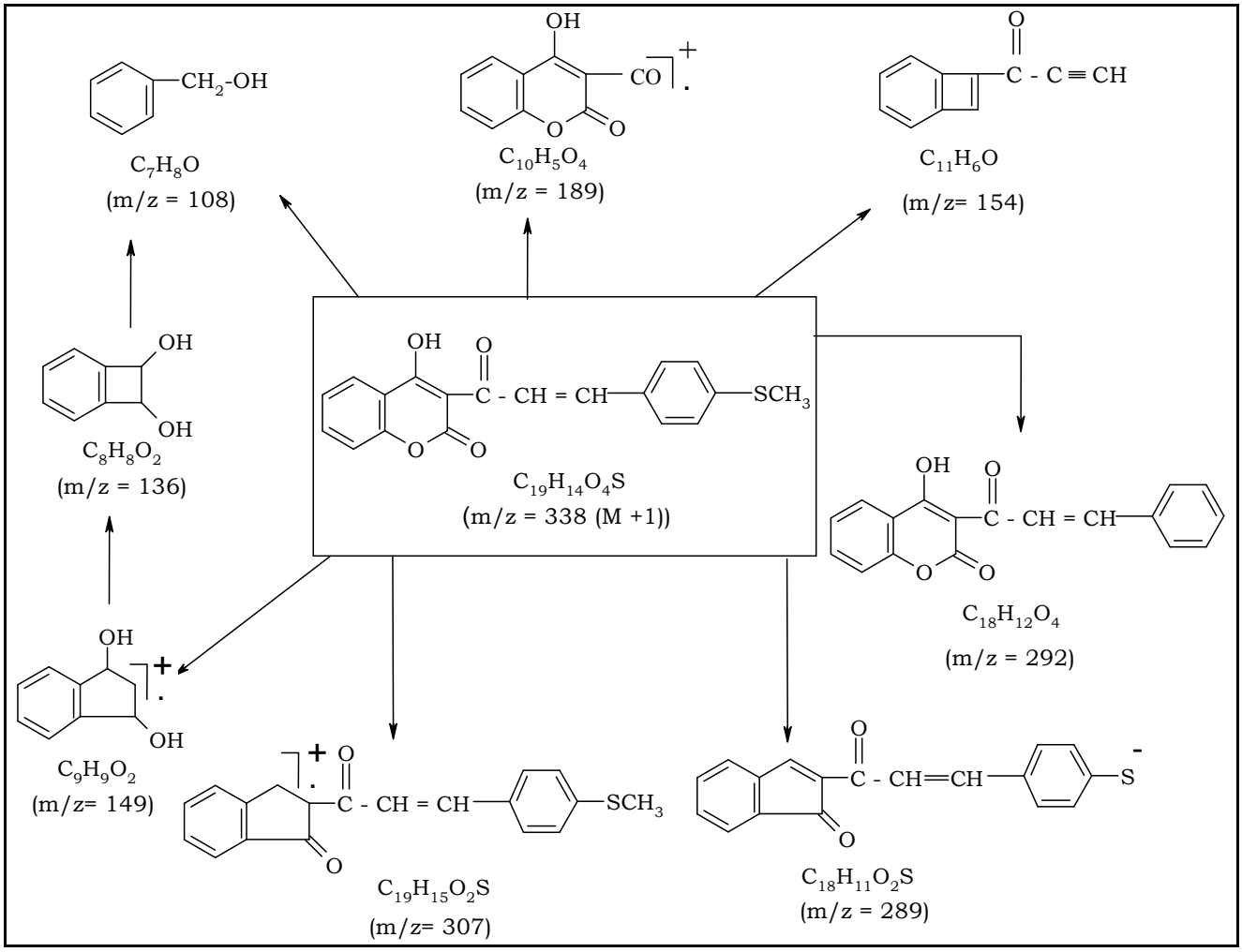
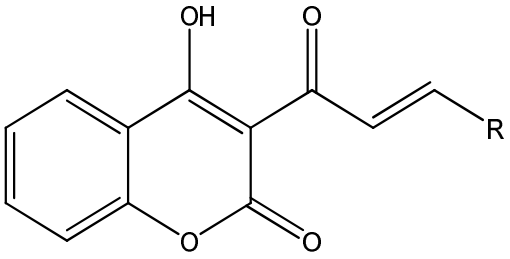


Table 3. Antimicrobial profile of some 4-Hydroxy-3-[3-(substitutedphenyl)prop-2-enoyl]-2H-chromen-2-ones



4-hydroxy-3-[3-(substituted phenyl)prop-2-enoyl]2h-chromen-2-ones

Sample Code	R	Antimicrobial Activity						
		Antibacterial			Antifungal			
		Staph. Aureus 209p	E.coli ESS 22.31	Aspergillusfumigatus	Candida albicans	Candida albicanes ATCC 10231	Candida krusei G03	Candida glabrata H05
1.	2.	3.	4.	5.	6.	7.	8.	9.
VCH-1	3-methoxy-4-hydroxy phenyl	19	-	-	-	-	-	-
VCH-2	4-thio methyl phenyl	-	-	-	-	-	-	-
VCH-4	2-nitro phenyl	21	-	-	-	-	-	-
VCH-7	5-N,N-di methyl Phenyl	-	-	-	-	-	-	-
VCH-8	3-nitro phenyl	10	-	-	-	-	-	-
VCH-9	2-hydroxy phenyl	-	-	-	-	-	10h	-
VCH-11	3-bromo phenyl	18	-	17	11	-	-	-
VCH-12	2,5-di chloro phenyl	2h	-	-	-	-	-	-
VCH-13	2,4-di chloro phenyl	14	15h	-	-	-	-	-
VCH-18	3-indoyl	-	-	-	12h	10	-	-

Antifungal activity (Chavda *et al*., 2002; Synthesis and biological, 2003; 33)

Aspergillusfumigatus, Candida albicans, Candida albicans ATCC 10231, Candida krusei G03, Candida glabrata H05 were employed for testing antifungal activity using well method. The culture was maintained on sabaroud's agar slants. Purified compound were used for testing the fungicidal activity. The medium was inoculated with 72 hours old, 0.5 ml suspension of different fungal spores in separate flasks. About 25 ml of the inoculated medium was poured and evenly spread in sterilized petridish and allowed to stand for 24 hours. The wells (10 mm in diameter) were formed by the help of a sterile borer in agar medium of sterile 0.1 ml pipette. The plates were incubated at 370 C for 24 hours. After completion of the incubation period the zones of inhibition of growth were measured.
[*Important Note*:

Solvent used: 10 % DMSO in methanol.Concentration used: 1 mg/ml well/disc).

Control Used: Fluconazole.(-) Denotes no activity.(h) Indicates hazy.**]**

Table 3 shows the comparative study of 10 homologues. The results are in tabular form [Table 3].Compound 1, 4, 8, 12 and 13 proved active against *S. aureus 209p*. Compound 11 have shown its activity against bacterial strain *S. aureus 209p*and fungal species*Aspergillusfumigatus, Candida albicans*. This indicates the structure activity relationship (SAR) that when halogen occupies the meta position of phenyl ring of coumarinchalcone; a broad spectrum antimicrobial activity is favoured. Compound 18 have shown activity against *Candida albicansATCC10231*. Compound 2, 7 and 9 exhibited no promising activity. The anti S. aureus 209p activity was found tobe in the order of substituents :

2-NO_2> 3-OCH_3> 4-OH > 3-Br > 2, 4-di Cl > 3-NO_2

Conclusion

The alternative synthetic route adopted by us has been proved more advantageous than the reported one[28]. Out of the homologues studied for their antimicrobial activity 50 % were proved active against *S. aureus 209,* the structure activity relationship (SAR) was observed by the results of compound no. 11 that when halogen occupies the meta position of phenyl ring of coumarinchalcone; a broad spectrum antimicrobial activity is favoured. The anti *S. aureus 209p* activity was found to be in the order of substituents : 2-NO_2> 3-OCH_3> 4-OH > 3-Br > 2, 4-di Cl > 3-NO_2.

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