Improved Synthesis of Coumarin-Chalcone under ultrasonic irradiation and their *invitro* antibacterial and antioxidant activities

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Abstract

The 3-acetyl coumarins reacted with *p*-substituted benzaldehyde gave Coumarin-Chalcone compounds (3a-3g). The method used for the synthesis is ultrasonic irradiation, this method offered several advantages such as good yields and short reaction time than the conventional method.All the new compounds were characterized on the basis of the IR,[1]HNMR, [13]CNMR,Mass and elemental analysis. The synthesized compounds analysed for their antibacterial activity by two gram positive and two gram negative bacteria and antioxidant activity by DPPH method

Keywords: Coumarin, Ultrasonic; Antibacterial, Antioxidant, DPPH method

INTRODUCTION

Ultrasound irradiation has been increasingly used in organic synthesis in last three decades. A large number of organic reactions can be carried out in higher yield, shorter reaction time and milder conditions under ultrasonic irradiation[1]. As a part of our interest is the synthesis of Coumarin-chalcone, which can be modified into different types of heterlcycles such as Pyramidines and Pyrazolines.

In recent years ,Coumarin derivatives have received significant attention owing to their diverse range of biological properties such as antiviral,anticoagulant[2],antibacterial[3],antifungal[4], anti HIV[5], and antihistamine[6] actions. Besides the wide biological applications of coumarin and its derivatives the chemical literature also embodies their some applications from the material view point such as cosmetics, optical brightening agents, dispersed fluorescent and laser dyes.[7]In addition, some coumarins are of interest because of their toxicity, carcinogenity and photodynamic effects.[8]

Chalcones, precursors of open chain flavonoids and isoflavonoids present in edible plants, and their derivatives have attracted increasing attention due to numerous potential pharmacological applications. They have displayed a broad spectrum of pharmacological activities. Changes in their structure have offered a high degree of diversity that has proven useful for the development of new medicinal agents having improved potency and lesser toxicity

In view of this, we design and synthesize a series of novel Coumarin-chalcone derivatives with the aim of finding novel coumarin derivatives with more excellent properties. Synthesized routes are shown in Scheme I.The structures of the new compounds were deduced basedon

IR,^{1}H NMR spectroscopy and elemental analysis.

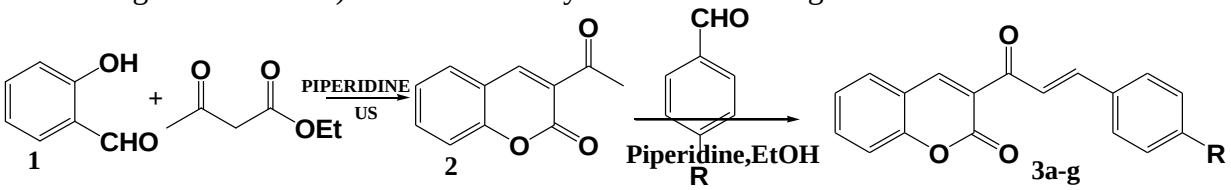
EXPERIMENTAL

General instrumentation procedures

The^{1}H and^{13}CNMR spectra were recorded on anINOVA-400 NMR spectrometer, using $CDCl_3$as solvent and TMS as internal reference (chemical shifts in). The IR spectra were recorded as KBr pellets on a Bruker Equinox 55 FT-IR spectrophotometer. Melting points were measured on a Yanaco MP-S_3 melting point apparatus and are uncorrected. Elemental analyses were performed on a Thermo Flash EA1112 elemental analyzer. Analytical thin layer chromatography (TLC) was performed on silica gel GF254(Qingdao, China) with EtOAc and light petroleum (fraction boiling in the range of 60-90 °C) and detection by UV light or iodine vapor. Sonication was performed by Fisher sonicator (with a frequency of 25 kHz and a nominal power 600 W). All reagents were commercial products of analytical grade and were used directly without processing unless otherwise specified.

General procedure

Sonicated reactions: To an equimolar mixture of 3-acetylcoumarin 2(1 g, 5.3 mmoles) and substituted benzaldehyde (5.3mmoles) was added piperidine (0.2mL, 1.64 mmoles) drop wisely with continuous stirring until homogeneity was achieved. The mixture was subjected to ultrasound irradiation at room temperature. After the completion of reaction as indicated by TLC (EtOAc-petroleum ether = 1:1). The crude product was filtered, dried, and recrystallized from appropriate solvent to afford 3a–g .



R=H,,OH,OCH$_3$N(CH$_3$)$_2$NO$_2$,F,Cl

3-Acetylcoumarin (2). To a mixture of salicyaldehyde(0.86 mL, 81.89 mmoles) and ethyl acetoacetate (11.5mL,90.13 mmoles) was added catalytic amount of piperidine (0.2mL, 1.64 mmoles) and swirled thoroughly. The mixture was sonicated for 5 min. The solid product was filtered, dried, and recrystallized from methanol to afford pure 3-acetyl coumarin1in appropriate yield (Table 1). FTIR (KBr): 2925 (CH aliphatic), 1746 (C¼O ester), 1685 (C¼O), 1606 (C¼C), 1369cm.1H-NMR (CD3OD, 400 Hz):d2.27 (s, 3H, CH3), 7.42–7.84 (m, 4H, Benzofused coumarin-H),8.57(s, 1H,CoumarinH).^{13}C-NMR (CD3OD, 400 Hz):d 198.7 (C¼O), 159.4(C¼O), 153.0, 137.4, 131.2, 128.3, 127.9, 125.4, 118.1, 116.1,29.6 (CH_3) ppm. MS: m/z 188 (M^+, 80%), 145 (100%), 94(50%).

3-Cinnamoyl-2H-chromen-2-one (3a). Reagents: Compound 2(1.0g,5.3 mmoles), benzaldehyde (0.5 mL, 5.3 mmoles), piperidine (0.2 mL). Conditions: ultrasound irradiation for 20 min, at room temp. Purification: recrystallization (ethanol). FTIR (KBr): 1740, 1673, 1606, 1363cm-1.1H-NMR (CD_3OD, 400 Hz):d7.03 (d, 1H,J¼8.5 Hz,COACH¼C), 7.33–7.84 (m, 9H, Benzofused coumarin-4H

and Ar-5H), 7.82 (d, 1H,J¼8.5 Hz, COAC¼CH), 8.57 (s,1H, Coumarin-H).13C-NMR (CD3OD, 400 Hz): d 183.7(C¼O), 159.4 (C¼O), 153.0, 147.2, 142.2, 135.2, 134.2,128.6, 128.6, 128.5, 128.5, 128.3, 127.9, 127.9, 125.4, 125.4,118.1, 116.1 ppm. MS: m/z 276 (M^+, 75%), 199 (60%), 173(100%).

3-(3-(4-Hydroxyphenyl)acryloyl)-2H-chromen-2-one(3b). Reagents:Compound 2(1.0g,5.3 mmoles), *p*-hydroxybenzaldehyde (0.65 g, 5.3 mmoles), piperidine (0.2 mL). Conditions: ultrasound irradiation for 35min at room temp. Purification: recrystallization (ethanol).FTIR (KBr): 3241,1734, 1685, 1612, 1375 cm-1.^{1}H-NMR (CD3OD, 400 Hz):d 5.35 (s, 1H, OH, D_2O exchangeable), 6.65 (d, 2H, Ar-H), 7.03(d, 1H, J¼8.5 Hz, COACH¼C), 7.42–7.84 (m, 4H, Benzofused coumarin-H), 7.56 (d, 2H, Ar-H), 7.82 (d, 1H, J¼8.5Hz, COAC¼CH), 8.57 (s, 1H, Coumarin-H).^{13}C-NMR(CD3OD, 400 Hz):d 183.7, 159.4, 157.7 (CAOH), 153.0,147.2, 142.2, 134.2, 130.6, 130.6, 128.3, 127.9, 127.8, 125.4,125.4, 118.1, 116.1, 115.8 ppm. MS: m/z 292 (M+, 40%), 275(75%), 199 (50%), 145 (100%).

3-(3-(4-methoxyphenyl)acryloyl)-2H-chromen-2-one (3c). Reagents:Compound 2 (1.0 g, 5.3 mmoles), p-methoxybenzaldehyde (0.65 g, 5.3 mmoles), piperidine (0.2 mL). Conditions: ultrasound irradiation for 25min at room temp. Purification: recrystallization (methanol).FTIR (KBr): 2924 (Ar, C-H str), 1707(C=O str), 1633 (C=O str, α, β-unsaturated,cyclic), 1604 (C=C str), 1454 (alkane, C-H str), 1340 (C-O str).1HNMR (CD_3OD, 400 Hz):d5.35 (s, 1H, OH, D2O exchangeable), 6.70 (s, 1H, Ar-H), 6.83 (d, 1H, Ar-H), 7.03 (d, 1H,J¼8.8 Hz, COACH¼C), 7.16 (d, 1H, Ar-H), 7.42–7.84 (m, 4H,Benzofused coumarin-H), 7.53 (t, 1H, Ar-H), 7.96 (d, 1H,J¼8.8 Hz, COAC¼CH), 8.57 (s, 1H, Coumarin-H).13C-NMR(CD_3OD, 400 Hz):d 183.7 (C¼O), 159.4 (C¼O), 158.4,153.0, 147.2, 142.2, 135.4, 134.2, 130.0, 128.3, 127.9, 125.4,125.4, 121.1, 118.1, 117.6, 116.1, 115.1 ppm. MS: m/z 292(M+, 30%), 275 (50%), 199 (25%), 145 (100%).

3-(3-(4-Dimethylaminophenyl)acryloyl)-2H-chromen-2-one(3d). Reagents: Compound 2 (1.0 g, 5.3 mmoles), 4-(N,Ndimethylamino)benzaldehyde (1.27 g, 5.3 mmoles), piperidine(0.2 mL). Conditions: ultrasound irradiation for 30min at room temp. Purification: recrystallization (ethanol). FTIR (KBr): 1746, 1685, 1594,1375 cm-1.1H-NMR (CD_3OD, 400 Hz):d 3.06 (s, 6H,2xCH3), 6.71 (d, 2H, Ar-H), 7.03 (d, 1H, J ¼8.5 Hz,COACH¼C), 7.72 (d, 2H, Ar-H), 7.42–7.84 (m, 4H, Ar-H),7.82 (d, 1H,J¼8.5 Hz, COAC¼CH), 8.57 (s, 1H, Coumarin-H).^{13}C-NMR (CD_3OD, 400 Hz):d 183.7,159.4, 153.0, 150.3, 147.2, 142.2, 134.2, 129.7, 129.7, 128.3,127.9, 125.4, 125.4, 124.7, 118.1, 116.1, 111.7, 111.7, 41.3(2xCH3) ppm. MS: m/z 319 (M+, 70%), 199 (100%).

3-(3-(4-Nitrophenyl)acryloyl)-2H-chromen-2-one (3e) Reagents:Compound 2 (1.0g,5.3mmoles),*p*-nitrobenzaldehyde (0.80g,5.3 mmoles), piperidine (0.2 mL). Conditions: ultrasound irradiation for 25min at room temperature. Purification: recrystallization (methylated spirit). IR (KBr): 1740, 1673, 1606, 1364, 738 cm^{-1}.^{1}H-NMR (CD_3OD, 400 Hz):d7.32 (d, 1H,J¼8.5 Hz, COACH¼C), 7.96 (d, 1H,J¼8.5Hz, COAC¼CH), 7.42–7.84 (m,

4H, Benzofused coumarinH), 8.03 (d, 2H, Ar-H), 8.21 (d, 2H, Ar-H), 8.57 (s, 1H, Coumarin-H).13C-NMR (CD_3OD, 400 Hz):d183.7 (C¼O), 159.4(C¼O), 153.0, 147.2, 147.1, 142.2, 141.3, 134.2, 129.0, 129.0,128.3, 127.9, 125.4, 125.4, 123.8, 123.8, 118.1, 116.1 ppm.MS: m/z 321 (M+, 70%), 275 (47%), 145 (100%).

3-(3-(4-Fluorophenyl)acryloyl)-2H-chromen-2-one(3f).

Reagents:Compound 2(1.0g,5.3 mmoles), *p*-fluorobenzaldehyde (0.74g, 5.3mmoles), piperidine (0.2 mL). Conditions: ultrasound irradiation for 30min at room temperature. Purification: recrystallization (methanol). IR (KBr): 1718 and 1666 (C=O), 1612 (C=C), 1184.21 (C-O-C).^{1}HNMR (CD_3OD, 400 Hz): d 7.03 (d, 1H, J ¼8.5 Hz,COACH¼C), 7.82 (d, 1H,J¼8.5 Hz, COAC¼CH), 7.44 (d,2H, Ar-H), 7.68 (d, 2H, Ar-H), 7.42–7.84 (m, 4H, Benzofused coumarin-H), 8.57 (s, 1H, Coumarin-H).^{13}C-NMR (CD_3OD,400 Hz): d 180.5, 159.6, 157.8, 147.6, 145.2, 143.9, 142.6, 138.9, 133, 132.5, 131.9, 130, 129.9, 129.1, 125.9, 125.3, 124.2, 120.3ppm. MS: m/z 294.7 (M+, 80%), 260 (30%).

3-(3-(4-Chlorophenyl)acryloyl)-2H-chromen-2-one (3g).

Reagents:Compound 2(1.0g,5.3 mmoles), *p*-chlorobenzaldehyde (0.74g, 5.3 mmoles), piperidine (0.2 mL). Conditions: ultrasound irradiation for 40min. at room temp. Purification: recrystallization (methanol). IR (KBr): 1728.10 and1685.67 (C=O), 1558.38 (C=C), 1107.06 (C-O-C).1HNMR (CD3OD, 400 Hz): d 7.03 (d, 1H, J ¼8.5 Hz,COACH¼C), 7.82 (d, 1H,J¼8.5 Hz, COAC¼CH), 7.44 (d,2H, Ar-H), 7.68 (d, 2H, Ar-H), 7.42–7.84 (m, 4H, Benzofused coumarin-H), 8.57 (s, 1H, Coumarin-H).13C-NMR (CD3OD,400 Hz): d 183.7, 159.4, 153.0, 147.2, 142.2, 134.2, 133.5,133.3, 129.0, 129.0, 128.7, 128.7, 128.3, 127.9, 125.4, 125.4,118.1, 116.1 ppm. MS: m/z 310.5 (M+, 80%), 275 (40%).

Biological Evaluation of Synthesized Compounds:-

A) Antimicrobial activity:-

Determination of Zone of Inhibition:

The Zone of Inhibition was determined by the disk diffusion method[9]. The method is as follows-

Preparation of nutrient agar media (500ml):- Peptone 5gm, Sodium chloride 2.5gm, Beef extract 5gm, Agar10gm, Distilled water q.s., Adjusted pH 7.2-7.4. Sodium chloride, peptone, beef extract ,agar were weighed out and dissolved in required amount of distilled water by keeping the media in the steam bath, agar was melted out and the indicator was added and the volume was made upto distilled water , ph was adjusted at 7.2-7.4 . Then the flask was plugged and wrapped in paper then autoclave at 15 PSI pressure at 1210 c at 15 min.

Preparation of Liquid broth media:- Peptone 5gm, Sodium chloride 2.5gm, Yeast extract 2.5gm, Distilled water P^H 7.2-7.4. Sodium chloride, peptone, yeast extract were weighed out the dissolve in required amount of distilled water by keeping the media in the steam bath, and the volume was made with the distilled water, ph was adjusted at 7.2-7.4 .Then the flask was plugged and wrapped in paper and then autoclave at 15 PSI pressure at 1210 c for 15 min.

Procedure:-

The test was performed according to the disk diffusion method[9] adopted with some modification for the prepared compound using streptomycin as reference. The prepared compounds were tested against one strain of Gram +ve bacteria, Gram –ve bactria, fungi. Whatman filter paper disk of 5mm diameter were sterilized by autoclaving for 15 min at 121^{0}C. The sterile disk were impregnated with different compounds (600gm/disk). Agar plates were surface inoculated uniformly from the both culture of the tested microorganism. The disk were placed on the medium suitably spaced apart on the plate were incubated at 50 c for 1 hr to permit good diffusion and then transferred to an incubator at 37 c. for 24hr for bacteria and 280 c for 72hrs for fungi. The inhibition zones caused by the various compounds on the microorganism were explained. The antibacterial data shown in the **Table-2**

Antioxidant activity:-

Antioxidant potential: It is well-known that free radicals cause autoxidation of unsaturated lipids in food. In addition, antioxidants are known to interrupt the free-radical chain of oxidation and to donate hydrogen from phenolic hydroxy groups, thereby, forming stable free radicals, which do not initiate or propagate further oxidation of lipids[10] .The antioxidant potential of any compound can be determined on the basis of its scavenging activity of the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical as described by Sadhu et al.[11]DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. The aliquot of the different concentrations (5-500 µg/mL) of the test sample is added to 3 ml of a 0.004% ethanolic solution of DPPH. Absorbance at 517 nm is determined after 30 min, and IC (Inhibitory concentration 50%) is also determined. IC50 value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals. The formula used for %inhibition is as follows:

%inhibition= (BlankOD – SampleOD/BlankOD) ×100

Sampling, screening and IC calculation: At first 5 test tubes were taken to make aliquots of 5 different concentrations level (5, 10, 50, 100 and 500µg/mL). Tested sample and ascorbic acid were weighed 3 times and dissolved in ethanol to make the required concentrations by dilution technique. DPPH was weighed and dissolved in ethanol to make 0.004% (w/v) solution. To make homogeneous solutions of the tested samples, magnetic stirrer was used. After making the desired concentrations, 2 ml of 0.004% DPPH solution was applied on each test tube by using pipette. The room temperature was recorded and kept the test tubes for 30 minutes in light exposure to complete the reactions. DPPH was also applied on the blank test tubes at the same time where only ethanol was taken as blank. After 30 min, absorbance of each test tube was determined by UV spectrophotometer. Then %inhibitions were plotted against log concentration of each of the test sample. Then IC was calculated from the graph. The experiment was performed in duplicate and average absorption was noted for each concentration. Ascorbic acid was used as a positive control. Calculated antioxidant data of all the test samples were summarized in **Table 3.**

Free radical scavenging mechanism of antioxidants: The 1,1-diphenyl-2-picrylhydrazyl radical has been widely used to evaluate the free radical scavenging capacity of different antioxidants[12-14].With this method it is possible to determine the antiradical power of an antioxidant activity by measurement of the decrease in the absorbance of DPPH at 517 nm. Resulting from a color change from purple to yellow the absorbance decreased when the DPPH is scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH molecule. In the radical form this molecule had an absorbance at 517 nm, which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic spin paired molecule . The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color.The color turns from purple to yellow as the molar absorptivity (optical density) of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with hydrogen radical from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to the number of electrons captured

RESULTS AND DISCUSSION

The ultrasonic irradiation method for the synthesis of Coumarin-Chalcones give high yield and their physicochemical properties are shown in Table 1. The reaction time for the reactants differed based on the functional groups present on them and their electronic effects. The reaction time was reduced considerably from hours to minutes in the ultrasound irradiation method

Table-1 Physicochemical properties of synthesized compounds (1–2g)

Comp no.	Molecular formula	Mol. wt	Reaction time (min)	Yield (%)	M.P. (C	Rf [a]	Color	Elem. Anal. %Calcd. (%Found) C	H	N
2	$C_{11}H_8O_3$	188	5	92.6	119–121	0.52	yellow	70.2(70.1)	4.3(4.4)	–
3a	$C_{18}H_{12}O_3$	276	20	97.0	142–143	0.50	yellow	78.3(78.1)	4.3(4.5)	–
3b	$C_{18}H_{12}O_4$	292	35	97.8	246-249	0.68	Yellow	74.0(73.8)	4.1(4.3)	–
3c	$C_{19}H_{14}O_3$	290	25	92.5	161-163	0.54	Yellow	78.6(78.3)	4.8(4.5)	–
3d	$C_{20}H_{17}NO_3$	319	30	81.6	217-218	0.71	Red	75.2(74.9)	5.3(5.0)	4.4(4.7)
3e	$C_{18}H_{11}NO_5$	321	25	78.3	170-172	0.69	orange	67.3(67.2)	3.4(3.2)	4.4(4.6)
3f	$C_{18}H_{11}FO_3$	304	30	95.1	172-174	0.66	Cream	78.9(78.6)	5.3(5.1)	–
3g	$C_{18}H_{11}ClO_3$	310	35	77.1	222-223	0.59	Yellow	69.7(69.4)	3.5(3.7)	–

Antibacterial activity

The invitro antibacterial activity against different strains of gram positive bacteria (S. aureus and P. aeruginosa), gram negative bacteria (E. coli, K. pneumoniae).The antibacterial activity against all the bacterial strains was significant when compared with the standard, sulfamethoxazole. All the compounds showed good antibacterial activity against P.aeruginosa at all the tested concentrations. The results of the

antibacterial activities are tabulated in Table 2.

Table-2 Result of antibacterial screening (sensitivity testing) on bacteria with zones of Inhibition (in mm).

Comp.	***Gram Positive bacterias***						***Gram negative bacterias***					
Conc(µg/ml)	***Staph aureus***			***P. aeruginosa***			***Escherichia coli***			***K. pneumoniae***		
	Zone of inhibitions(mm)											
	50	100	200	50	100	200	50	100	200	50	100	200
IIIa	12	13	16	14	15	16	12	14	15	13	14	16
IIIb	11	12	15	16	16	17	10	12	15	10	12	15
IIIc	11	14	14	10	13	15	10	10	11	8	11	14
IIId	13	15	17	12	14	18	7	13	15	5	8	9
IIIe	12	13	15	11	15	15	11	13	14	9	10	11
IIIf	14	16	18	10	14	15	13	11	11	12	14	14
IIIg	16	18	20	10	13	16	13	14	14	10	13	15
Std	15	16	20	12	13	15	`15	18	21	10	14	18

Table-3 Observation for antioxidant activity in terms of DPPH method

Compound code	**% Scavenging**					**IC50 ug/ml**
	25ug/ml	**50ug/ml**	**75ug/ml**	**100ug/ml**	**125ug/ml**	
IIIa	14.61	22.11	25.41	37.95	49.12	128.17
IIIb	26.00	51.11	63.03	74.80	84.00	45.75
IIIc	36.20	57.21	66.21	73.45	81.14	42.21
IIId	19.16	29.26	32.61	51.27	61.20	94.31
IIIe	11.32	24.35	38.25	55.51	64.71	91.42

IIIf	18.27	28.57	41.32	52.51	56.46	93.24
IIIg	15.12	33.11	42.54	51.91	61.28	95.30
STD (Ascorbic acid)	22.28 5ug/ml	41.03 10ug/ml	52.06 15ug/ml	75.02 20ug/ml	96.10 25ug/ml	15.10

CONCLUSIONS

An efficient and eco-friendly process for the synthesis of coumarine-chalcones was developed in the laboratory. The ultrasound reaction resulted in the higher yields and significant reduction in the reaction time.Many synthesized compounds depicted antibacterial activity higher than that of sulfamethoxazole against gram positive as well as gram negative bacteria and many showed activity equivalent to sulfamethoxazole.All the compounds showed good antibacterial activity against P. aeruginosa.From the invitro antioxidant activity it was found that all the compound shows potent antioxidant activity and compound 3c shows maximum potency.

Acknowledgement

We want to acknowledge our profound thanks to Director of Asthagiri Herbal Research Foundation providing all the facilities.Our sincere thanks is expressed to CLRI, Chennai for spectral analysis

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