WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 7, 1157-1166. Research Article ISSN 2277– 7105

DESIGN AND PHARMACOLOGICAL STUDIES OF NEW COUMARIN DERIVED CHALCONE SYNTHESIZED COMPOUNDS

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Article Received on
09 Feb. 2018,

Revised on 28 Feb. 2018,
Accepted on 21 Mar. 2018,

DOI: 10.20959/wjpr20187-11657

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ABSTRACT

New Coumarin derived Chalcones (**COU 1&2 and COU C1-6)** have been synthesized from condensation of resorcinol and ethylacetoacetate. The newly synthesized compounds were characterized by IR, ^{1}H NMR and MASS spectral data. These compounds were tested for their Antioxidant, Anti-inflammatory and Antimicrobial activities. Among all compounds, **COU C5** & **COU C6** showed good anti microbial and antifungal activites due to presence of hydroxy group**. COU C2** and **COU C5** showed significant anti-inflammatory activity due to hydroxyl and nitro group.

KEYWORDS: Coumarin Derived Chalcones, Antioxidant, Anti inflammatory, Antioxidant.

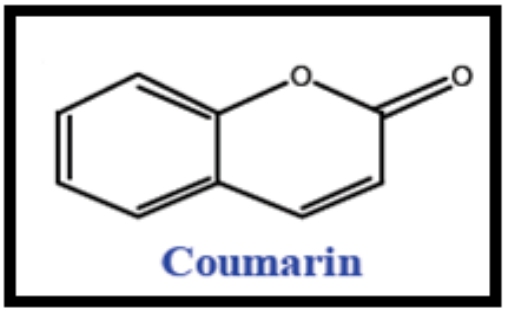
INTRODUCTION

Historically benzopyrans have been known as chromans, chromanones, chromones, 2- and 3-chromenes. The coumarins are the largest class of 1-benzopyran derivatives.[1] Coumarin chemically known as 2H-1-benzopyran-2-one. They are found mainly in higher plants. Most natural coumarins are oxygenated at C-7; Umbelliferone (7-hydroxycoumarin) being regarded as the structural and biogenetic parent of the more highly oxygenated coumarins.[2] Several methods were developed for the synthesis of coumarins, such as the Pechmann reaction.[3] Perkin reaction,[4] Knoevenagel reaction[5] and Reformastsky reactions.[8] Among these, the Pechmann reaction has been the most widely used method. Coumarins possess distinct biological activity and considerable medical interest, due to their antibacterial[9] anticoagulants,[10] antibiotic.[11] antifungal,[12] anticancer,[13] anti-inflammatory,[14] antiHIV[15] and antioxidant[16] properties. The 4-methyl coumarin had been demonstrated to possess a multiple biological activity. The amino group is an effective substitute for the hydroxyl group for antioxidant property and lipid peroxidation inhibition. Ortho dihydroxy and ortho

hydroxy-amino coumarins were found to possess highest antioxidant and radical scavenging activities.

COUMARIN

Coumarin (benzopyrones) is a compound containing two rings of six Members heterocycle rings with two oxygen atoms. Classification of Coumarins includes simple coumarin, furanocoumarins, Pyranocoumarins and coumarins substituted in the pyrone ring 4. Simple coumarins are compound that undergoes hydroxylation, Alkoxylation and alkylation to form its derivatives. For furanocoumarins, These compounds consist of five-members furan ring attached to the Coumarin concleus. pyranocoumarins is a coumpound that containied a Linear or angular type with substituents on benzene and pyrone rings 5.



EXPERIMENTAL

The following experimental methods were used for the characterization of the synthesized compounds. Melting points of the synthesized compounds were determined in open capillary tubes and are uncorrected. IR spectra was recorded on ELICO FTIR spectrometer using potassium bromide pellets. ^{1}H-NMR spectra of the compounds in deutiriated dimethyl sulfoxide was recorded on BRUKER Av 400 spectrometer. Mass spectra was recorded on GCMS QP 5000 shimadzu. Thin layer chromatography was performed using precoated aluminium plates coated with silica gel GF254[E.Merck]. n-hexane: ethylacetate in the ratio of 6:4 was used as the fluent. The spots were visualized in the ultraviolet light chamber.

SYNTHETIC METHOD

Synthetic method was carried out by three steps:

Step 1: Synthesis of 7-hydroxy-4-methylcoumarin(COU 1)

80 ml of Sulphuric acid was kept in a ice cold temperature (0-50°C). Mixture of resorcinol (22g, 0.2 mole) and ethyl aceto acetate (26g, 0.2 mole) was added to ice cold sulphuric acid

with constant stirring which was confirmed by TLC. The mixture was kept at room temperature overnight and poured over crushed ice. The solid obtained was filtered, washed with water and recrystallised from ethanol.

Step 2: Synthesis of 6-acetyl-7-hydroxy-4-methylcoumarin(COU 2)

Freshly fused and powdered zinc chloride (13.629 g, 0.1 mole) was dissolved in glacial acetic acid (12 ml) by heating in beaker on a sand bath. Dry compound 7-hydroxy-4-methyl coumarin **(COU 1,** 14.608 g, 0.083 mole) was added with stirring to the mixture at 1400 °C to obtained compound 6-acetyl-7-hydroxy-4-methyl coumarin **(COU 2)** which was dried and recrytallised.

Step 3: Synthesis of Coumarin derived Chalcones (COU C1-6)

To a mixture of 6-acetyl-7-hydroxy-4-methylcoumarin **(COU 2)** (0.01 mol) and the appropriate aromatic aldehyde (0.01 mol) in methanol was added a solution of 40% sodium hydroxide in distilled water with constant stirring of the reaction flask.. To the reaction mixture 2N HCl was added, washed with water and crystallized from ethanol to furnish the desired product **(COU C1-6).**

RESULTS AND DISCUSSION

Initially various catalysts chosen for the model reaction using resorcinol and ethylacetoacetate at ambient temperature were studied. Among them conc.H_2SO_4 at ice cold temperature(0-50 °C) was found to be the best in terms of yield and reaction time. Similarly various catalysts are used for acetylation reaction. Among them reaction with glacial acetic acid in presence of zinc chloride was found to be best. The reaction proceeded with good yield.

PHARMACOLOGICAL STUDIES OF SYNTHESIZED COMPOUNDS

Anti-inflammatory studies[17]

The synthesized compounds COU C1-6 were evaluated for anti-inflammatory activity by carrageenan induced Paw oedema method using Indomethacin as standard drug. Among the twelve compounds, compound COU C2 and COU C5 at 100mg/kg (PO) showed significant reduction in Paw oedema due to presence of hydroxy and nitro group at para position of the phenyl group. The anti inflammatory activity of the synthesized compounds by carrgeenan induced Paw oedema was tabulated.

ANTIMICROBIAL STUDIES[18]

The synthesized compounds were evaluated for *in vitro* antibacterial activity against various Gram-positive and Gram –negative bacteria's by disc diffusion method and their antifungal activity against Aspergillus flavus and Aspergillus niger at 100µg/ml. Compounds COU C 5, C6 & C7 possessed very significant Antibacterial and Antifungal activities compared with that of the standard Ciprofloxacin and Fluconozole.

ANTI OXIDANT ACTIVITY[19]

All the synthesized compounds COU C1-6 were evaluated for in vitro antioxidant activity by DPPH radical scavenging method and compared with that of the standard ascorbic acid at 517nm. The percentage inhibition of various concentrations of the synthesized compounds along with the standard was measured and was tabulated.

COU 1: 7-hydroxy-4-methylcoumarin

Molecular formula: $C_{10}H_8O_3$; IR 9(KBr) (cm^{-1}):3035.17(Ar-H), 1501.11(C=C), 1597.98(C=O), 1167.51(C-O-C); ^{1}H NMR (400MHz, DMSO-*d6*)(δ ppm): 2.43(s, 3H, CH3), 6.106-7.616 (m, 4H, Ar); Mass (m/z): 176, 177(100%) (M^{+1}).

COU 2: 6-acetyl-7-hydroxy-4-methylcoumarin

Molecular formula: $C_{12}H_{10}O_4$; IR (KBr*)* (*(*cm^{-1}): 2958.18(-C-H), 1385.72 (-OH), 1516(C=C) ^{1}H NMR (400MHz, DMSO-*d6*)(δ ppm): 2.427 (s,3H, CH3), 3.327-3.331(s, 3H,OCH3), 6.101-7.609 (m, 4H, Ar); Mass (m/z): 144, 174 (100%), 219(M^{+1}).

COU C 1: 6-cinnamoyl-7-hydroxy-4-methyl-2H-chromen-2-one

Molecular formula: $C_{19}H_{14}O_4$; IR (KBr*)* (*(*cm^{-1}): 1679.75(C=C), 1382.15(-OH), 1499.58 (C=O); ^{1}HNMR (400MHz, DMSO-*d6*) (δ ppm): 2.248 (*s*,3H,CH3), 6.824-6.845 (*dd*,2H,C=C), 6.941-7.966 (*m*,8H, Ar); Mass (m/z): 60, 85(100%), 307 (M^{+1}).

COU C 2: 7-hydroxy-6-(3-(2-hydroxyphenyl)acrylolyl)-4-methyl-2H-chromen-2-one

Molecular formula: $C_{19}H_{14}O_5$; IR (KBr*)* (*(*cm^{-1}):1620.39(HC=CH), 1354.2(-OH), 1459.66(C=O); ^{1}HNMR (400MHz, DMSO-*d6*)(δ ppm): 2.269(s,3H,CH3), 6.097 (dd,2H, HC=CH) 6.715-7.891 (m,7H,Ar), 10.845(s,1H,OH), 12.181 (s,1H, OH); Mass (m/z): 85(100%), 87,169, 323 (M^{+1}).

COU C 3: 7-hydroxy-6-(3-(2-methoxyphenyl)acrylolyl)-4- methyl-2H-chromen-2-one

Molecular formula: $C_{20}H_{16}O_5$; IR (KBr*)* (cm^{-1}): 1677.90(HC=CH), 1382.99(-OH), 2834.38 (-OCH3); ^{1}HNMR (400MHz, DMSO-*d6*)(δ ppm): 2.327 (s,3H,CH3), 3.384 (s,3H,CH3), 6.987- 6.992(d,2H, HC=CH) *J*= 2, 7.014-7.913 (m,7H,Ar), 9.882(s,1H, OH); Mass (m/z): 91, 121(100%), 267, 337 (M^{+1}).

COU C 4: 7-hydroxy-6-(3-(4-chlorophenyl)acrylolyl)-4-methyl-2H-chromen-2-one

Molecular formula: $C_{19}H_{13}ClO_4$; IR (KBr*)* (cm^{-1}): 1681.81(HC=CH), 759.12(-Cl), 1313.76(-OH); 1HNMR (400MHz, DMSO-*d6*)(δ ppm): 2.371 (s,3H,CH3), 6.710-6.785 (dd,2H,HC=CH) *J*=2.4, 6.785-8.319 (m,7H,Ar), 10.516 (s,1H, OH); Mass (m/z): 60, 132, 174(100%), 271, 341 (M^{+1}).

COU C 5: 7-hydroxy-6-(3-(4-nitrophenyl)acrylolyl)-4-methyl-2H-chromen-2-one

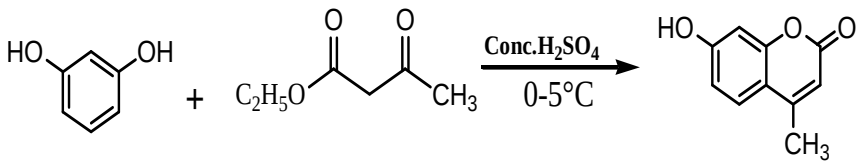
Molecular formula: $C_{19}H_{13}NO_6$; IR (KBr*)* (cm^{-1}): 1689.28(HC=CH), 1383.66(-NO2), 1342.62(-OH); ^{1}HNMR (400MHz, DMSO-*d6*)(δ ppm): 2.403 (s,3H, CH3), 6.573-6.594 (d,2H,CH=CH), 6.726-8.400(m,7H,Ar);Mass (m/z): 97, 131, 155(100%), 351 (M^{+1}).

COU C 6: 7-hydroxy-6-(3-(furan-2-yl)acrylolyl)-4-methyl-2H-chromen-2-one

Molecular formula: C17H12O5;IR (KBr*)* (cm^{-1}):1689.36(HC=CH), 1379.52(-OH), 1507.52(C=O);^{1}HNMR (400MHz,DMSO-*d6*)(δ ppm):2.429(s,3H, CH3), 6.106-6.118 (d,2H,CH=CH), 6.709-7.616(m,6H,Ar); Mass (m/z): 60, 149, 181(100%), 296 (M^{+1}).

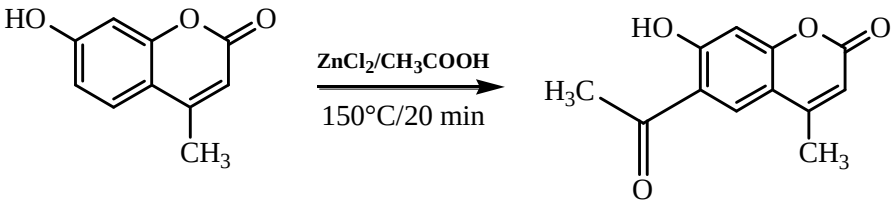
SYNTHETIC SCHEME

STEP-1



Resorcinol Ethylacetoacetate 7-Hydroxy-4-Methylcoumarin

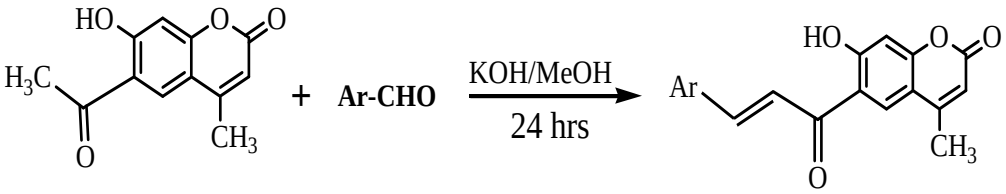
STEP-2



7-Hydroxy-4-Methylcoumarin

6-Acetyl-7-Hydroxy-4-Methylcoumarin

STEP-3



6-Acetyl-7-Hydroxy-4-Methylcoumarin

Aromatic Aldehyde

Coumarin derived chalcones

Table 1: Physical constants of the synthesized compounds.

Compound	Ar-CHO	M.Wt	Yield%	MP(°C)	R_f value
COU C 1	Benzaldehyde	306	24.50%	115°C -117°C	0.69
COU C 2	2-hydroxy Benzaldehyde	322	20.18%	230°C -232°C	0.52
COU C 3	2-methoxy Benzaldehyde	336	42.26%	160°C -162°C	0.81
COU C 4	4-chloro Benzaldehyde	340	51.41%	195°C -197°C	0.42
COU C 5	4-nitro Benzaldehyde	351	45.75%	120°C -122°C	0.64
COU C 6	Furan-2-carbaldehyde	296	52.25%	110°C -112°C	0.57

Table 2: Anti inflammatory Activity of the Synthesized Compounds Carragenan induced Paw Oedema.

Group	Dose mg/kg	Oedema volume and percentage oedema volume at			
		1st HOUR	2nd HOUR	3rd HOUR	4th HOUR
		MEAN±SEM %ROV	MEAN±SEM % ROV	MEAN±SEM	MEAN±SEM %ROV
Control	0.1ml	1.12±0.02	1.22 ±0.02	1.3 ±0.04	1.35 ±0.02
Standard Indomethacin	10	1.17 ±0.02 4.46%	1.02±0.02** 16.39%	0.93 ±0.02 28.46%	0.81 ±0.01** 40.00%
COU C 1	100	1.20 ±0.04 3.92%	1.17 ±0.02 14.53%	1.07 ±0.02* 21.98%	1.05 ±0.02** 34.65%
COU C 2	100	1.30 ±0.00 4.01%	1.17 ±0.02 15.67%	1.07 ±0.02** 19.98%	0.92 ±0.02** 31.85%
COU C 3	100	1.15 ±0.02	1.15 ±0.02	1.05 ±0.02*	0.95 ±0.02**

		2.67%	16.89%	20.75%	35.87%
COU C 4	100	1.17 ±0.04 4.46%	1.20 ±0.04 14.89%	1.12 ±0.02 18.93%	0.95 ±0.02** 29.62%
COU C 5	100	1.25 ±0.02 2.78%	1.15 ±0.02 8.76%	1.05 ±0.02** 19.23%	0.93 ±0.02** 31.11%
COU C 6	100	1.30 ±0.04 3.98%	1.20 ±0.04 10.43%	1.10 ±0.04* 15.67%	1.02 ±0.02** 20.74%

Table 3: Minimum inhibitory concentration of synthesized compounds against gram positive and gram negative bacteria.

Compound	Minimum Inhibitory Concentration			
	Microorganisms			
	Staphylococcus aureus	Bacillus subtilis	Escherichia coli	Klebsiella pneumonia
COU C1	47µg	45µg	47µg	47µg
COU C2	46µg	47µg	48µg	45µg
COU C3	48µg	48µg	44µg	48µg
COU C4	45µg	45µg	49µg	44µg
COU C5	48µg	46µg	46µg	48µg
COU C6	44µg	44µg	48µg	43µg

Table 4: Zone of inhibition of synthesized compounds against gram positive and gram negative Bacteria.

Compound	Inhibition zone diameter in mm											
	Bacillus subtilis			Staphylococcus aureus			Escherichia coli			klebsiella pneumonia		
	50 µg	100 µg	150 µg	50 µg	100 µg	150 µg	50 µg	100 µg	150 µg	50 µg	100 µg	150 µg
COU C-1	06	08	12	06	09	11	07	09	14	07	10	14
COU C-2	05	07	10	08	10	13	06	08	12	09	11	13
COU C-3	06	09	13	07	09	12	07	09	14	06	10	13
COU C-4	08	10	11	06	07	10	06	08	12	05	07	09
COU C-5	07	09	13	08	10	12	07	08	13	06	08	13
COU C-6	05	08	12	07	09	12	08	10	13	07	09	13
Ciprofloxacin	15			15			15			15		

Table 5: Minimum inhibitory concentration of synthesized compounds against Aspergillus Flavus and Aspergillus Niger.

Sample code	Minimum Inhibitory Concentration	
	Microorganisms	
	Aspergillus Flavus	Aspergillus niger
COU C1	97µg	95µg
COU C2	96µg	97µg
COU C3	98µg	98µg
COU C4	95µg	95µg
COU C5	98µg	96µg
COU C6	94µg	94µg

Table 6: Zone of inhibition of synthesized compounds against Aspergillus Flavus and Aspergillus Niger.

Sample code	Inhibition zone diameter in mm			
	Aspergillus Flavus		Aspergillus Niger	
	100µg/ml	150 µg/ml	100 µg/ml	150 µg/ml
COU C-1	08	15	08	14
COU C-2	07	16	09	13
COU C-3	06	10	07	11
COU C-4	08	13	07	11
COU C-5	09	15	09	12
COU C-6	08	11	10	14
Fluconazole (100µg/ml)	15		16	
Control(DMSO)	-	-	-	-

Table 7: Antioxidant activity of the synthesized compounds by DPPH radical scavenging method.

Compound	%Radical scavenging activity Concentrations mg/ml					
	5µg/ml	10µg/ml	25µg/ml	50µg/ml	100µg/ml	IC 50 µg/ml
COU C-1	8.55	16.14	28.05	47.78	61.02	5.29
COU C-2	15.5	22.15	38.78	46.10	79.12	4.63
COU C-3	10.71	20.40	39.87	50.11	59.64	5.08
COU C-4	11.58	19.77	37.98	56.89	77.38	4.55
COU C-5	22.45	45.31	75.33	81.62	92.23	3.23
COU C-6	22.6	47.83	76.08	84.95	85.80	3.17
Ascorbic Acid	19.52	27.96	54.82	74.86	93.51	3.56

SUMMARY AND CONCLUSION

The objective of the present work is to synthesize coumarin derived chalcones and study their anti-inflammatory, antibacterial, antifungal and antioxidant in particular. Thus an attempt has been made in this direction. All the synthesized compounds show characteristic absorption peaks in IR, 1HNMR spectra. Almost the synthesized compounds shows expected molecular ion (M+1) fragments in the Mass spectra.

As expected, coumarin derived chalcones exhibited anti-inflammatory activity in which some of the synthesized compounds shown highly significant activity as compared to the standard employed for the study. Among all compounds COU C2 (2-hydroxy group) and COU C5 (4-nitro group) showed significant activity.

Anti microbial studies were performed for all the synthesized compounds. The zone of inhibition of various concentrations of synthesized compounds against Gram-positive, Gram-Negative bacteria and various fungi were measured. The minimum inhibitory concentration

of the synthesized compounds against various bacteria and fungi were determined by agar streak dilution method. Among all compounds, COU C8 (4-hydroxy-3-methoxy group), COU C 12(p-hydroxy group) showed good anti microbial and antifungal activites.

Anti Oxidant studies were performed for the synthesized chalcone compounds by DPPH free radical scavenging assay. These results revealed that the DPPH radical scavenging activities of the compounds increase in concentration- dependent manner and reach a plateau at the concentration of 100 μM in most cases. Among all compounds, COU C 5(4-nitro group) and COU C6 (furfural group) have shown significant activity.

ACKNOWLEDGEMENTS

The Authors are very much thankful to the Department of Chemistry, Acharya Nagarjuna University, Guntur for providing research facility for doing this work.

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