SYNTHESIS AND BIOLOGICAL EVALUATION OF 2-PHENYL CHROMEN-4-ONES FOR THEIR ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY

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Summary

Claisen-Schmidt condensation reaction of aldehydes and ketones afforded intermediate chalcones, which were cyclized into final products as substituted 2-phenyl chromen-4-ones. The synthesized compounds were characterized by physico-chemical and spectral studies. All the synthesized eight compounds were screened for antioxidant activity by DPPH radical scavenging assay method and antibacterial activity against *S. aureus*, *B. subtilis* (gram +ve), *P. aeruginosa*, *E. coli*, (gram –ve) and antifungal activity against *C. albicans* and *A. niger*. All the synthesized compounds have shown moderate antioxidant activity while the compounds AFLV-4, -7, -8 exhibited considerable antimicrobial activity.

Key words: chromen-4-ones, antioxidant, antimicrobial.

Introduction

Flavanones (2,3-dihydro-2-phenyl-4H-1-benzopyran-4-ones and derivatives), possess saturated C-ring, is a type of flavonoids, are widely distributed in nature. They are considered as important chemical scaffolds due to their wide range of biological activities viz. antioxidant, antibacterial, antifungal, antitumoral, anti-inflammatory and hypotensive[1-2]. The traditional method for the synthesis of flavanones consists of an intramolecular conjugated addition of *o*-hydroxychalcones, to the corresponding cyclic carbonylic system.

This reaction can be performed using acids, silica gel, bases, light, heat, or electrons. The acid catalyzed cyclization can be carried out by refluxing the chalcone in acetic acid, or also in ethanol or in other suitable solvent, in the presence of an acid catalyst such as H_2SO_4 or H_3PO_4. Basic conditions are seldom used due to decomposition or retroaldol reaction[3-4]. The corresponding chalcones, are usually obtained by Claisen-Schmidt condensation of *o*-hydroxyacetophenones and benzaldehydes[5].

Experimental

Melting points were determined by using a melting point apparatus (Shital Scientific Industries, India) and were not corrected. Ultra-Violet spectra were obtained on Shimadzu UV 2450, Japan. Infrared spectra were obtained on a Shimadzu FTIR-8310 (Shimadzu, Japan) using potassium bromide discs. ^{1}H NMR spectra were recorded on a Bruker 400 MHz spectrophotometer (Bruker, USA). Chemical shifts are reported in parts per million (*d*) units relative to an internal standard of tetramethylsilane. Mass spectra were recorded on a GC-MS QP 5050 (Shimadzu, Japan). The purity of all compounds was established by single spot on the TLC plates (Merck, Germany). Iodine vapour was used as developing agent. The solvent system used was toluene: pet ether- 4:1.

Synthetic scheme

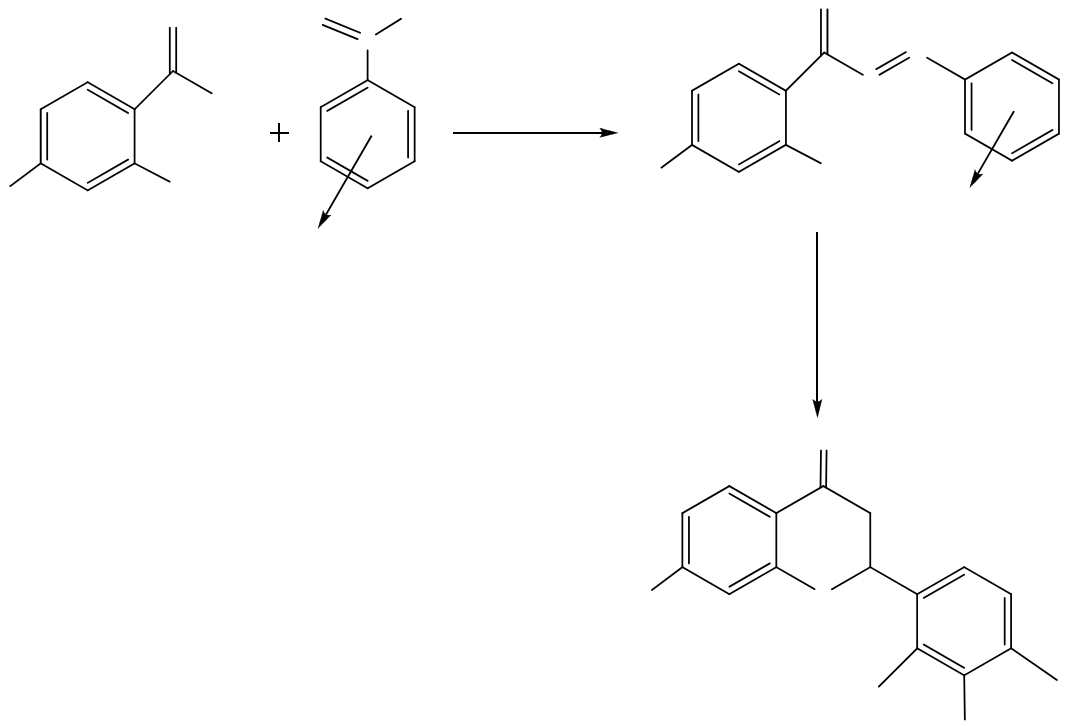

Table I. Synthesized 2-phenyl chromen-4-ones

Table II. Physico-chemical data of synthesized 2-phenyl chromen-4-ones

Compd.	X	R_1	R_2	R_3	Chemical Name
AFLV-1	H	H	H	OCH_3	2,3-dihydro-2-(4-methoxyphenyl)chromen-4-one
AFLV-2	H	H	H	Cl	2-(4-chlorophenyl)-2,3-dihydrochromen-4-one
AFLV-3	H	H	H	CH_3	2,3-dihydro-2-*p*-tolylchromen-4-one
AFLV-4	H	H	Cl	Cl	2-(3,4-dichlorophenyl)-2,3-dihydrochromen-4-one
AFLV-5	F	H	H	OCH_3	7-fluoro-2,3-dihydro-2-(4-methoxyphenyl)chromen-4-one
AFLV-6	F	OCH_3	H	H	7-fluoro-2,3-dihydro-2-(2-methoxyphenyl)chromen-4-one
AFLV-7	F	OCH_3	OCH_3	OCH_3	7-fluoro-2,3-dihydro-2-(2,3,4-trimethoxyphenyl)chromen-4-one
AFLV-8	F	H	H	Cl	2-(4-chlorophenyl)-2,3-dihydrochromen-4-one

[a] **Solvent system:** toluene: pet ether- 4:1

Compd.	Mol. formula	Mol. mass	M. p. (°C)	Yield (%)	R_F value[a]
AFLV-1	$C_{16}H_{14}O_3$	254.09	112-114	50	0.42
AFLV-2	$C_{15}H_{11}O_2Cl$	258.04	118-120	52	0.41
AFLV-3	$C_{16}H_{14}O_2$	238.10	108-110	53	0.43
FLV-4	$C_{15}H_{10}O_2Cl_2$	292.01	115-117	45	0.45
AFLV-5	$C_{16}H_{13}O_3F$	272.08	98-100	46	0.47
AFLV-6	$C_{16}H_{13}O_3F$	272.08	97-99	40	0.48
AFLV-7	$C_{18}H_{17}O_5F$	332.11	102-104	35	0.65
AFLV-8	$C_{15}H_{10}O_2ClF$	276.04	95-97	48	0.51

Synthesis of Chalcones

To a solution of aryl aldehyde (0.05 mol) and substituted *o*- hydroxyacetophenone (0.05 mol) in ethanol was added a solution of 10% sodium hydroxide. The reaction mixture was stirred at room temperature for 24 h. The progress of the reaction was monitored through TLC. After the desired time the reaction mixture was kept in ice chest for 2-3 h. The reaction mixture was then poured into a beaker containing crushed ice and was neutralized with dilute HCl. The desired chalcone obtained was washed with water and recrystallized from methanol.

Conversion of Chalcones to Flavanones

Synthesized chalcones were cyclized into flavanones by stirring suspension of chalcones in water with piperidine for 1 h (AFLV-1, -2, -3), refluxing with trifluoroacetic acid at 80 ˚C for 20 min to 1 h (AFLV-4), refluxing for 24 h with methanol-HCl (AFLV-5, -6, -7, -8) respectively[6]. The corresponding flavanones were separated from the chalcones by column chromatography after eluting with toluene: pet ether- 4:1.

Spectral data of AFLV-1: UV (λ Max., nm), (MeOH): 320.20, 281.00, 251.60. IR: (KBr), (ν cm^{-1}): 1679.90 (C=O, str.), 1172.60 (OCH_3, str.). ^{1}H NMR (DMSO.d6, δ in ppm): 5.41 (1H, dd, 2-H of chromen-4-one), 3.12 (1H, dd, 3-Ha of chromen-4-one), 2.87 (1H, dd, 3-Hb of chromen-4-one), 3.70 (3H, s, -OCH_3 of phenyl ring), 6.97-7.59 (8H, m, Ar-H). Mass: m/z (relative intensity %): 254 $[M]^+$ (33%), 134 $[C_9H_{10}O]^+$.

Spectral data of AFLV-2: UV (λ Max., nm), (MeOH): 322.60, 282.29, 254.62. IR: (KBr), (ν cm^{-1}): 1680.40 (C=O, str.). ^{1}H NMR (DMSO.d6, δ in ppm): 5.46 (1H, dd, 2-H of chromen-4-one), 3.05 (1H, dd, 3-Ha of chromen-4-one), 2.89 (1H, dd, 3-Hb of chromen-4-one), 6.99-7.49 (8H, m, Ar-H). Mass: m/z (relative intensity %): 263 $[M+1]^+$ (48%).

Spectral data of AFLV-3: UV (λ Max., nm), (MeOH): 317.16, 278.00, 253.59. IR: (KBr), (ν cm^{-1}): 1683.35 (C=O, str.).^{1}H NMR (DMSO.d6, δ in ppm): 5.45 (1H, dd, 2-H of chromen-4-one), 3.10 (1H, dd, 3-Ha of chromen-4-one), 2.90 (1H, dd, 3-Hb of chromen-4-one), 2.43 (3H, s, -CH_3 of phenyl ring), 6.92-7.54 (8H, m, Ar-H). Mass: m/z (relative intensity %): 238 $[M]^+$ (28%).

***In vitro* Antioxidant Studies**

***1, 1-Diphenyl-2-picrylhydrazyl radical scavenging assay*[7]**

0.5 mL of test compound or standard (2, 4, 8, 16, 32, 64, 128, 256, 512 and 1000 µg/mL) was added to 0.5 mL of DPPH solution in test tubes. Control test tubes were loaded with 0.5 mL of methanol and 0.5 mL DPPH. The tubes were incubated at 37 ^{o}C for 30 min without exposing to light and the absorbance of each solution was measured at 517 nm using Ultra-Violet spectroscopy. Experiment was performed in triplicate. Ascorbic acid was used as a standard. The percentage scavenging by test compounds at each concentration was calculated by using the formula: (control – test/control) × 100.

Antimicrobial activity[8-10]

All the synthesized compounds were screened for antibacterial and antifungal activity at 30 µg/mL by Kirby-Bauer disc diffusion method. The compounds were tested for antibacterial activity against two Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) species using antibiotic assay medium no.-1, seed agar while antifungal activity against *Candida albicans* and *Aspergillus niger* using sabouraud dextrose agar medium. Ciprofloxacin and Nystatin at 30 µg/mL were used as standard for antibacterial and antifungal activity respectively.

Results and discussion

Chalcones were synthesized by Claisen-Schmidt condensation reactions of various aryl aldehydes and *o*- hydroxyacetophenones, The cyclization of corresponding chalcones was carried out by refluxing the chalcone in presence of methanol-hydrochloric acid, piperdine, and trifluoro aceticacid yielded corresponding flavanones. Completion of reaction was checked by TLC solvent system, toluene: pet ether- 4:1 and final products were purified by column chromatography. Recrystallization was done by using methanol.

Synthesis of chalcones was confirmed by corresponding band-I at 300-320 and band-II between 240-260 nm in UV spectroscopy while synthesized final products as 2-phenyl chromen-4-ones were confirmed by UV, IR, NMR and Mass techniques. Compound AFLV-1 showed absorption maxima at 320.20, 281.00, 251.60 nm in UV spectroscopy, while IR spectrum showed peaks at ν = 1679.9 (C=O str), and 1172.6 (OCH_3 str) cm^{-1}. NMR spectrum of AFLV-1 exhibited characteristic double doublet peaks for 2-H, 3-Ha and 3-Hb protons at δ = 5.41, 3.12 and 2.87 ppm respectively. Mass spectrum of compound AFLV-1 showed corresponding molecular ion peak at 254 and base peak at 134 (m/z). This along with other physical and spectral data confirms the synthesis of compounds.

Among the compounds tested for antioxidant activity, compounds AFLV-3, -4, -5, -7, -8 showed the IC_{50} less than 250 µg/mL. AFLV-7 was found to be most active with IC_{50} 231.59 µg/mL. The other compounds have also shown the IC_{50} less than 300 µg/mL.

Table-III: Antioxidant activity of substituted 2-phenyl chromen-4-ones

S. No.	Compd.	IC_{50} ±SEM[a] (µg/ mL)
1	AFLV-1	295.92±2.13
2	AFLV-2	277.05±1.14
3	AFLV-3	249.82±3.43
4	AFLV-4	238.88±1.18
5	AFLV-5	231.79±2.37
6	AFLV-6	260.00±1.69
7	AFLV-7	231.59±2.10
8	AFLV-8	247.42±2.39
9	Ascorbic acid	12.64±1.28

a- average of three determinations

All the synthesized eight 2-phenyl chromen-4-ones were screened for their antibacterial activity against two Gram positive (*S. aureus* and *B. subtilis*) and two Gram negative (*E. coli* and *P. aeruginosa*) species. Among the compounds tested, AFLV-4, -6, -7 showed the moderate antibacterial activity. Substitution of choloro group at 3rd and 4th position of phenyl ring in AFLV-4 enhanced the antibacterial activity while substitution of fluoro group at 7th

position of chromen-4-one ring and methoxy groups at the 2, 3, and 4th positions of phenyl ring exhibited moderate antibacterial activity. When we substituted the chromen-4-one ring with fluorine atom at 7th positions and chloro group at the 4th positions of phenyl ring (AFLV-8), registered the highest antibacterial activity among the synthesized compounds.

Table-IV: Antimicrobial activity of substituted 2-phenyl chromen-4-ones

S. No.	Compd. (30µg/ml)	Zone of inhibition (mm)					
		S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*	*C. albicans*	*A. niger*
1	AFLV-1	-	10	-	-	8	9
2	AFLV-2	-	11	-	10	10	11
3	AFLV-3	-	-	-	-	-	-
4	AFLV-4	10	15	8	10	9	10
5	AFLV-5	-	-	-	-	-	-
6	AFLV-6	-	-	-	-	-	-
7	AFLV-7	11	12	9	9	10	11
8	AFLV-8	12	14	10	12	11	11
9	Ciprofloxacin	28	31	30	28	-	-
10	Nystatin	-	-	-	-	24	20

Conclusion

The Claisen-Schmidt condensation of various aryl aldehydes and *o*- hydroxyacetophenones afforded intermediate chalcones which were further cyclized to give various substituted 2-phenyl chromen-4-ones. Among the compounds tested for antioxidant activity, compound AFLV-7 was found to be most active, with IC_{50} 231.59 µg/mL. The substitution of methoxy groups at 2, 3 and 4th postions of 2-phenyl ring with fluoro group at 7th position of chromen-4-one moiety showed highest antioxidant activity (AFLV-7). It was seen that presence of fluoro group at 7th position of chromen-4-one moiety and methoxy groups at various positions of 2-phenyl ring increased the antioxidant activity viz. (AFLV-5, -6, -7, -8). Presence of chloro groups at 3 and 4th position of 2-phenyl ring retains the antioxidant activity even though fluoro group was absent at 7th position of chromon-4-one ring. Our preliminary screening suggested that further *in vitro* and *in vivo* studies should be taken up to explore the free radical scavenging potential of 2-phenyl chromen-4-ones.

All the synthesized compounds were screened for antimicrobial activity. The presence of fluoro group at 7th position of chromen-4-one moiety, have shown the improved antimicrobial activity (AFLV-7, -8), though 3, 4-dichloro substituted phenyl ring in AFLV-4

retains the antimicrobial activity. Hence it can be concluded that presence of halogen atoms either on the chromen-4-one moiety or on 2-phenyl ring of chromen-4-one increases the antimicrobial activity, though further study is required to come up with the noteworthy conclusion.

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References

1. Lee JI, Gung Jung MG, Jung HJ. A Novel synthesis of flavanones from 2-hydroxybenzoic acids. *Bull. Korean Chem Soc* 2007;28(5):859-62.
2. Adibi AM, Ahmad F, Idris MS. Synthesis and antimicrobial activity of 4′,5,7-trihydroxy-3′-prenylflavanone. *J Chem Sci* 2008;120(5):469-73.
3. Sagrera GJ, Seoane GA. Microwave accelerated solvent-free synthesis of flavanones. *J Braz Chem Soc* 2005;16(4):851-56.
4. Wang Y, Tan W, Li WZ, Li Y. A facile synthetic approach to prenylated flavanones: First total syntheses of (±)-bonannione A and (±)-sophoraflavanone A. *J Nat Prod* 2001;64(2):196-99.
5. Wang X, Cheng S. Solvent-free synthesis of flavanones over aminopropyl-functionalized SBA-15. *Catal Commun* 2006;7(9):689-95.
6. Reddy GJ, Latha D, Thirupathaiah C, Rao KS. A mild, one-pot synthesis of 3-cyano-4-benzopyrones from 2-hydroxyacetophenones. *Tetrahedron Lett* 2004;45(4):847-48.
7. Mensor LL, Menzes FS, Leitao GG, Reis AS, dos Santos TC, Coube CS, Leitao SG. Screening of Brazilian extract for antioxidant activity by the DPPH free radical method, *Phytother Res* 2001;15(2):127-30.
8. Amin R, Krammer B, Abdel-Kader N, Verwanger T, El-Ansary A. Antibacterial effect of some benzopyrone derivatives. *Eur J Med Chem* 2010;45(1):372-78.
9. Srinivasan KK, Neelima Y, Joseph A, Sreejith G, Ciraj AM, Rao JV. Synthesis of novel furobenzopyrone derivatives and evaluation of their antimicrobial and antiinflammatory activity. *Indian J Pharmaceutical Sc* 2007;69(2):326-31.
10. Khan KM, Saify ZS, Khan MZ, Hayat S, Begum S, Noor F, Choudhary MI, Perveen S, Atta-Ur-Rahman. Synthesis, antifungal, and phytotoxic effects of some benzopyrone derivatives. *Nat Prod Res* 2004;18(1):21-27.