

Design, Synthesis and Biological Evolution of Substituted 3-Chloroflavones

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Abstract: The present protocol provide an easy and practical approach for the synthesis of flavones in good yield. The formal procedure does not require further purification like, chromatography. This method is green and ecofriendly and economically beneficial. Structures were confirmed by IR, C¹³H¹NMR and Mass spectra. The prepared derivatives screened for their biological activities and most of them exhibiting antimicrobial and antibacterial activity.

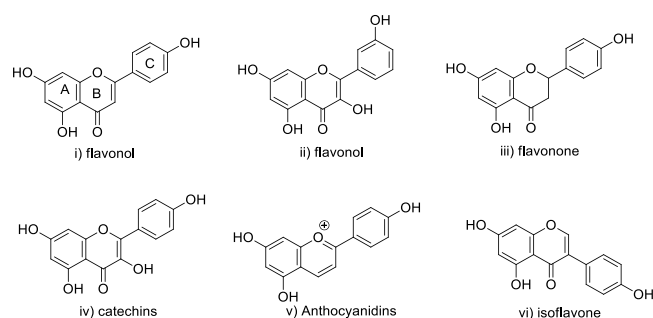
Index Terms: Flavones, Antibacterial, Antimicrobial, green and Ecofriendly.

I. INTRODUCTION

Flavonoides are ubiquitous and wide in plant kingdom and privilege class of heterocycles. They consist of three rings (A, B, C) and can be divided into six group (Flavones, Flavonols, Flavanones, Catechins, Anthocyanidins and Isoflavones.) depending on degree of oxidation of the ring C, hydroxylation pattern and the substitution at the third position¹ as shown in fig. 1.1. Flavones are owing to plethora of pharmacological activities and valuable precursor for the synthesis of biologically active derivatives. Flavones and flavonols possess greater antiproliferative activity than chalcones and flavonones². Other derivatives of flavones exhibiting various biological activities in mammals like inhibition of microbial and xanthine dehydrogenase inhibitory activity.³ The most important one is being antioxidant activity.⁴ It is observed that even a high intake of flavonoids in dietary is safe and do not have any side effect.⁵ Flavonoids act as potent inhibitors of molecular targets and showed a significant role in pharmaceutical effect, including antimicrobial,⁶⁻⁷ anticancer,⁸⁻¹¹ efficient in chemotherapy,¹²⁻¹³ antimutagenic,¹⁴ estrogenic, estrogen receptor binding,¹⁵⁻¹⁷ antiulcer,¹⁸⁻¹⁹ anti-inflammatory,²⁰ 3-methoxy and 4-hydroxy group are showing potent antiviral activity.²¹

Several methods exist for flavones synthesis, including Claisen Schmidt condensation and oxidative coupling,²² Wittig reaction,²³ Baker Venkataraman rearrangement,²⁴ Wacker oxidation process,²⁵ Ganguly's synthesis.²⁶ In recent developments such dehydrative cyclization involves the use of catalysts (Pd (OAc)₂, Ag(OAc),²⁷ Si-Supported reactions,^{28,29} CuCl₂³⁰) etc. and oxidizing agents³¹ ([Dichloro Dicyano benzoquinone](#) (DDQ), Diphenyldisulfane (Ph-S-S-Ph,^{19,20}) I₂ in Dimethyl sulphoxide (DMSO),²¹ I₂ and SiO₂,²² I₂ and Al₂O₃,²³ and InBr₃,²⁴). In our previous work flavones were synthesized by traditional methods.^{3, 32, 33} However, contents were required heat at 140°C for 3 h in oil bath. Yield of these reactions are often moderate and usually gives mixture of products and its separation require lot of solvent.³⁴ Washing required 10% sodium thiosulfate solution for several times. Sometimes DMSO is used to facilitate the reaction but it gets decomposed at high temperature and reduce yield. So we used alternative method for the synthesis of flavones, in this method we used catalyst CuCl₂ in Poly Ethylene Glycol (PEG)-400 as a green solvent,^{35, 36} this synthetic route give pure and high yield product. The products obtained by this procedure are easily isolated by recrystallization.

Fig. 1.1 Classification of flavonoids



II. MATERIALS AND METHODS

The melting points of prepared derivatives were recorded in the liquid paraffin bath using open end capillaries and are found to be uncorrected. Thin Layer Chromatography (TLC) was used to check the purity and completion of reaction. IR spectra of compounds were scanned on FTIR Perini Elmer model RXI spectrometer. ¹H NMR spectra recorded in DMSO-d₆ and CDCl₃ on Avance-300 MHz spectrometer by (DOP-13 A) operator procedure using TMS as an internal standard (chemical shift is given in δ ppm). The mass spectra (MS) were recorded on EIMS 40-600 DA mass spectrometer and the GCMS solution system.

General procedure for the synthesis of 3, 6-dichloro-2-(3-(4-hydroxyphenyl)-1-phenylpyrazol-4-yl) chromen-4-one (3a).

To a solution of 1-(5-chloro-2-hydroxyphenyl)-3-[3-(4'-hydroxyphenyl)-1-phenyl-1 H-pyrazol-4-yl]-2propen-1-one (2a) (0.465 g; 1 m mole) in PEG-400 (15 ml), 1.0 g CuCl₂ was added. Contents were refluxed under mild condition for 2 hours. After completion of the reaction, monitor by (TLC), the reaction mixture cooled at room temperature and poured in ice cold water (100 ml), obtained product was filtered and washed with ice cold water. The product was recrystallized from ethanol, to get desired product **3a**. All the remaining derivatives were prepared by the same experimental procedure.

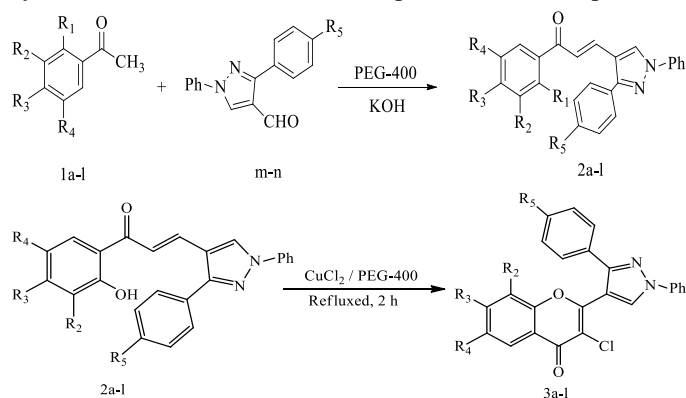


Table-1 Synthesized Derivatives of 3-chloroflavones (3a – 3l)

Sr. No.	Flavones	Substituents			
		R ₂	R ₃	R ₄	R ₅
1	3a	H	H	Cl	OH
2	3b	Br	H	Cl	OH
3	3c	I	H	Cl	OH
4	3d	I	H	CH ₃	OH
5	3e	I	CH ₃	H	OH
6	3f	H	H	Cl	Cl
7	3g	Br	H	Cl	Cl
8	3h	I	H	Cl	Cl
9	3i	H	OH	H	Cl
10	3j	H	CH ₃	Cl	Cl
11	3k	Cl	OH	Cl	Cl

12	3l	I	CH ₃	Cl	Cl
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1)3,6-dichloro-2-(3-(4-hydroxyphenyl)-1-phenyl-1H-pyrazol-4-yl)-4H-chromen-4-one (3a):

IR (KBr, ν, cm⁻¹): 3070.5(Ar-OH), 1647.1(Ar-C=O), 1533.3 and 1450.4 (Ar-CH=CH), 1338.5 (C=N), 1095.5 (C-O-C), 748.3 cm⁻¹(Ar-Cl);¹HNMR (300 MHz, DMSO):δ 7.30-8.20(m, 11H, Ar-H), 9.10 (s, 1H, H-5 of pyrazole), 12.70(s, 1H, Ar-OH).Mass (MS)-m/z; 449.14 [M⁺] Chemical formula C₂₄H₁₄O₃N₂Cl₂; C¹³NMR (PPM Rel. to TMS): 107.7 (C-Cl), 113.0 (Pyrazole C₂), 119.1 (C-R₂), 129.0 (C-R₄), 130.3 (Pyrazole C₃), 136.4 (C-R₃), 150.4 (Pyrazole C₁), 158.5 (C-R₅), 178.0 (C=O), Anal. Calcd. For C₂₄H₁₄O₃N₂Cl₂: C-64.12; H-3.10; Cl-15.80; N-6.20% Found; C- 64.14; H-3.11; Cl- 15.81; N- 6.23%.

2)3,6-dichloro-2-(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-4H-chromen-4-one (3f):

IR (KBr, ν, cm⁻¹): 3070.5(Ar-OH), 1657.7 (Ar-C=O), 1596.9 (Ar-C=N), 1554.5, 1504.4 and 1442.7 (Ar-C=C), 1321.1, 1226.6(C-O-C), 759.9 (Ar-Cl) cm⁻¹; ¹HNMR (300 MHz, DMSO): δ 7.30-8.10(m, 12H, Ar-H), 9.25 (s, 1H, H-5 of pyrazole);Mass (MS)-m/z; 467.62 [M⁺] Chemical formula C₂₄H₁₃O₂N₂Cl₃, C¹³NMR (PPM Rel. to TMS): 107.7 (C-Cl), 113.0 (Pyrazole C₂), 119.1 (C-R₂), 129.0 (C-R₄), 130.3 (Pyrazole C₃), 134.3 (C-R₅), 136.4 (C-R₃), 150.4 (Pyrazole C₁), 178.0 (C=O); Anal. Calcd. For C₂₄H₁₃O₂N₂Cl₃: C- 61.60; H- 2.76; Cl-22.77; N- 5.96% Found; C- 61.60; H- 2.78; Cl- 22.78; N- 5.98%.

3)3,6-dichloro-2-(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-8-iodo-4H-chromen-4-one (3h):

IR (KBr, ν, cm⁻¹): 3100 (Ar-OH), 1631.7 (Ar-C=O), 1598.9 and 1508.2 (Ar-CH=CH), 1336.6, 1209.3(C-O-C), 1093.6, 754.1 (Ar-Cl) cm⁻¹; ¹HNMR (300 MHz, DMSO): δ 7.40-8.30(m, 11H, Ar-H), 9.35 (s, 1H, H-5 of pyrazole).Mass (MS)-m/z; 593.51 [M⁺] Chemical formula C₂₄H₁₂O₂N₂Cl₃I; C¹³NMR (PPM Rel. to TMS): 90.7 (C-R₂), 107.7 (C-Cl), 113.0 (Pyrazole C₂), 130.3 (Pyrazole C₃), 130.6 (C-R₄), 134.3 (C-R₅), 144.3 (C-R₃), 150.4 (Pyrazole C₁), 178.0 (C=O);

Anal.Calcd. For C₂₄H₁₂O₂N₂Cl₃I: C- 48.50; H- 2.00; Cl-39.30; N- 7.70% Found; C- 48.52; H- 2.02; Cl- 39.34; N- 7.71%.

I) 4)3,6-dichloro-2-(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-8-iodo-7-methyl-4H-chromen-4-one(3l):

IR (KBr, ν, cm⁻¹): 2181.3 (Ar-CH₃), 1647.1 (Ar-C=O), 1566.1 and 1527.5 (Ar-CH=CH), 1338.5, 1220.9(C-O-C), 758 (Ar-Cl) cm⁻¹; ¹HNMR (300 MHz, DMSO):δ 2.65 (s, 3H, Ar-CH₃), 7.40-8.10(m, 10H, Ar-H), 9.25 (s, 1H, H-5 of pyrazole);Mass (MS)-m/z; 607.53 [M⁺] Chemical formula C₂₅H₁₄O₂N₂Cl₃I; C¹³NMR (PPM Rel. to TMS): 15.0 (C-R₃), 92.7 (C-R₂), 107.7 (C-Cl), 113.0 (Pyrazole C₂), 130.3 (Pyrazole C₃), 130.6 (C-R₄), 134.3 (C-R₅), 135.5, 150.4 (Pyrazole C₁), and

178.0 (C=O); Anal. Calcd. For $C_{25}H_{14}O_2N_2Cl_3I$: C- 49.35; H- 2.00; Cl- 38.46; N- 4.60 % Found; C- 49.38; H- 2.30; Cl- 38.49; N- 4.62%.

III. RESULTS AND DISCUSSION

The derivatives of 3-chloroflavones were prepared by green and environmental being general procedure used in 3.2 and conformed by qualitative tests, elemental analysis and spectral analysis. The experimental and calculated percentage of C, H, X and N shows the difference between experimental and calculated percentage is very less within experimental error it indicates flavones derivatives were synthesized in stoichiometric proportion.

The newly synthesized derivatives of 3-chloroflavones were tested for antimicrobial activities against bacterial and fungal pathogens of plants and animals. 3-chloroflavones (**3a**, **3f**, **3j**, **3l**) are tested on the bacteria *Escherichia coli* (E coli), *Salmonella typhi* (St), *Staphylococcus aureus* (Sa) and *Bacillus subtilis* (Bs) found to be highly antifungal than used standard drug like Nystatin, specially these synthesized flavones are more antifungal against *Trichoderma viridae*, *Penicillium chrysogenum*, *fusarium moniliforme*, *microsporium cannis* and *candida albicans* fungi.

In the present work we synthesized the new derivatives of 3-chloroflavone (**3a-3l**) by using green and reusable solvent with high yield (85-96%) and screening for their biological activity. The obtained compound **3b**, **3f**, **3j** and **3l** found to be more active against *Trichoderma viridae*, *Penicillium chrysogenum*, *fusarium moniliforme*, *microsporium cannis* and *candida albicans* fungi than standard drugs penicillin, tetracycline and nystatin also from the other newly synthesized derivatives of flavones.

Table-2. Antimicrobial activity of flavone on Bacteria (Zone of inhibition in mm)

Ec = *Escherichia coli*,
St = *Salmonella typhi*,
Pv = *Proteus vulgaris*,
Sa = *Staphylococcus aureus*,
Pa = *Pseudomonas aeruginosa*,
Bs = *Bacillus subtilis*,
Bm = *Bacillus megaterium*,
Sm = *Serratia Marcescens*
NA = Not applicable
++ = More spores are affected,
± = Trace activity

Table-3. Antimicrobial activity of flavone on Fungi germination (Zone of inhibition in mm)

Entry	Fungi germination						
	An	Tv	Af	Pc	Fm	Mc	Ca
f1	++	25	++	11	15	±	15
f2	++	27	++	10	25	15	13
f3	++	23	++	11	15	10	–
f4	++	20	++	13	–	16	20
f5	++	22	++	10	–	10	–
f6	++	16	++	15	10	–	±
f7	++	25	++	±	10	++	±
f8	++	20	++	15	15	++	++
f9	++	18	++	12	10	15	++
f10	++	20	++	10	12	16	++
f11	++	18	++	±	10	12	++
f12	++	18	++	++	++	10	++
f13	++	25	++	10	12	8	15
f14	±	16	±	12	++	++	
f15	++	25	++	12	12	8	13
f16	++	18	++	16	10	10	12
f17	++	18	++	12	12	10	12
control (DMSO)	±	++	±	±	±	±	±
Penicillin	NA	NA	NA	NA	NA	NA	NA
Tetracycline	NA	NA	NA	NA	NA	NA	NA
Nystatin	14	18	14	17	10	10	17

An = *Aspergillus niger*
Tv = *Trichoderma viridae*
Af = *Aspergillus flavus*
Pc = *Penicillium chrysogenum*,
Fm = *Fusarium moniliforme*,
Mc = *Microsporium cannis*
Ca = *Candida albicans*
NA = Not applicable
++ = More spores are affected,
± = Trace activity

CONCLUSION

In the present work we synthesized the new derivatives of 3-chloroflavone (**3a-3l**) by using green and reusable solvent with high yield (85-96%) and screening for their biological activity. The structure of obtained 3-chloroflavones derivatives were confirmed by spectral data. The obtained compound **3b**, **3f**, **3j** and **3l** found to be more active against *trichoderma viridae*, *Penicillium chrysogenum*, *fusarium moniliforme*, *microsporium cannis* and *candida albicans* fungi than standard drugs penicillin, tetracycline.

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