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A Novel Catalytic Synthesis of Flavones under Autoclave Conditions and Comparative Study of Anti-cancer Activity

K. Rajeshbabu[1], S. Pushpalatha[2], B. Ramakrishna[3] and V. Madhavarao[1*]

[1]Department of Chemistry, Bapatla Engineering College (autonomous), Bapatla, Acharya Nagarjuna University, 522101, India.
[2,3]St. Marys Pharmacy College, Deshmukhi, Hyderabad, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author KR performed the main experimental work and study; author SP involved in biological evaluation; author BR involved in literature gathering and author VM is our mentor, designed and managed entire study. All authors read and approved the final manuscript.

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ABSTRACT

Novel, less toxic, more effective catalysts were used to synthesize various flavones under auto clave conditions. Substituted o- hydroxyl acetophenones and benzaldehydes were reacted in-presence of 2, 4, 6-trimethylpyridine, a pinch of $TiCl_4$ and yielded chalcones. Equimolar ratio of KIO_3, KI, and H_2SO_4 was used as *in situ* catalytic source for I_2 to convert chalcones to flavones. The progress of the reactions was monitored by TLC. Crude compounds were recrystallized from methyl alcohol. We obtained good yields of products within less time periods. We had gone through IR, HNMR, Elemental analysis and mass data to characterize the compounds. Selective compounds were screened for cytotoxic activity against A549 human tumour cell lines by MTT method and comparative study has been done.

**Corresponding author: Email: krbchem@gmail.com, vmrgpm@gmail.com;*

Keywords: Autoclave conditions; 2, 4, 6- tri methyl pyridine; KIO_3; flavones; cytotoxic activity.

1. INTRODUCTION

Flavones are natural heterocyclic compounds containing pyrone ring bearing aromatic moieties and having waste diversity in substitutions. The name Flavone came from the Greek literature *flavs,* which means yellow color.

Flavones show wide range of biological activities including anti-bacterial [1], anti-fungal [2,3], anti-cancer [4,5], HIV- inhibitory [6], analgesic [7,8], anti-osteoporotic [9], anti-diabetic [10], cardio vascular [11], neurodegenerative [12].

Synthesis of Flavones are well precursored by chalcones and thereby cyclisation. Preparation of chalcones was catalyzed by various organic/inorganic bases and acids; and was cyclized by the catalysis of I_2/DMSO, diphenylmercuptoperoxide and dichlorodicayano-quinone.

Due to their great biological importance; we focused on various reagents for synthesis. Pyridine also was used as 3° base for the preparation of chalcones, but due to its toxic effect we have chosen alkyl substituted pyridine. They poses low vapors due to their high boiling points and more basic than un substituted pyridine due to +I effect. Catalytic amount of $TiCl_4$ was added to make active methyl group-more active. More eco-friendly and effective catalytic source 'KIO_3 was used with equimolar ratio of KI and H_2SO_4' to convert chalcone to flavone.

2. MATERIALS AND METHODS

All chemicals used in our experimental work were obtained commercially from SVR chemical suppliers, Hyd. The progress of the reactions was monitored by TLC using silica gel-G (Merck grade) and U.V.light for detection of spots. The structures of compounds were characterized by m.p. s, 1H-NMR, mass spectral data. All the m.p.s were determined in open capillaries using electrothermal m.p. apparatus and uncorrected. 1H-NMR spectral data was collected on Wormhole-v nmrs 400 MHz spectrometer in DMSO-d6 using TMS as an internal reference.

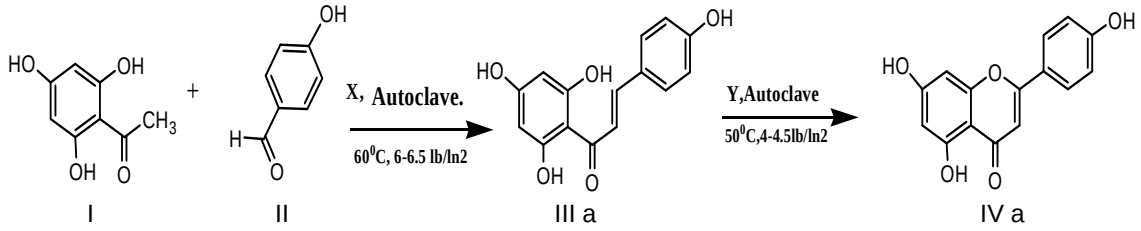
2.1 General Procedures

2.1.1 Synthesis of Chalcone III a

Compound 1 (0.01 m.mol), catalytic amount of $TiCl_4$ were charged in reaction container and stirred for 10min. 3 ml of 2, 4, 6-tri methyl pyridine, Compound 2 (0.01 mol) was added drop wise. The contents were kept in autoclave and heated in Muffle furnace at 60°C 6-6.5 lb. /ln^2 pressure for 2 – 2.5 hrs. The reaction progress was monitored by TLC using 8:2 n-hexane, ethyl acetate. The mixture was poured in crushed ice, neutralized using 0.1N HCl, yellow ppt was separated, dried and recrystallized from methyl alcohol.

2.1.2 Synthesis of Flavone IV a

Equimolar ratio of KIO_3, KI, H_2SO_4 were taken in a conical flask and contents were stirred for 20 min. Chalcone (0.01 m.mol) is dissolved in ethyl alcohol and added to the catalyst, contents are kept in autoclave, heated in Muful furnace at 50℃, 4-4.5lb/ln^2 pressure for 20 min. Contents were poured in crushed ice, precipitate is filtered out, washed with hyposolution to remove excess of iodine, dried and recrystallized from ethyl alcohol.



Scheme-1

X = 2, 4, 6-trimethyl pyridine, $TiCl_4$; Y = KIO_3, KI, H_2SO_4

2.2 Spectral Data

2.2.1 5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one (IVa)

IR (KBr) Cm^{-1}: 3463, 3137, 1242, 1633, 687;

^{1}H-NMR (400 MHz, DMSO-d6): δ 12.67 (1H, S H-5), 10.61 (1H, S H-7), 8.85 (1H, S H-4'), 8.08-7.59 (4H, m H-2', 3', 5', 6'), 7.56 (1H, S H-8), 6.92 (1H, S H-6), 6.64 (1H, S H-3).

Elemental analysis: Calculated: C, 66.67; H, 3.73; O, 29.60., Found: C, 66.64; H, 3.71; O, 29.63;

ESIMS: *m/z: 270.9 (M^+)*

2.2.2 5, 7-dihydroxy-2-phenyl-4H-chromen-4-one (IVb)

IR (KBr) Cm^{-1}: 3610, 3136, 1609, 1244, 689;

H^1-NMR (400 MHz, DMSO-d6): δ 12.97 (1H, S H-5), 10.42 (1H, S H-7), 6.94-6.77(7H, m H-6, 8, 2', 3', 4', 5', 6'), 6.37 (1H, S H-3).

Elemental analysis: Calculated: C, 70.86; H, 3.96; O, 25.17., Found: C, 71.20; H, 3.92; O, 25.25.

ESIMS: mass *m/z*: 254.9 (M^+).

2.2.3 5, 6, 7-trihydroxy-2-phenyl-4H-chromen-4-one (IVc)

IR (KBr) Cm^{-1}: 3016, 2925, 1629, 1215, 666;

H^1-NMR (400 MHz, DMSO-d6): δ 12.97 (1H, S H-5), 10.85 (1H, S H-6), 10.38(1H, S H-7), 7.94 (1H, S H-8), 6.97-6.50(5H, m, H-2', 3', 4', 5', 6'), 6.20 (1H, s H-3).

Elemental analysis: Calculated: C, 66.67; H, 3.73; O, 29.60., Found: C, 66.63; H, 3.71; O, 29.63.

ESIMS: *m/z* 271.0 (M^+).

2.2.4 5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-4H-chromen-4-one (IVd)

IR (KBr) Cm^{-1}: 3018, 2924, 1629, 1215, 750;

H^1-NMR (400 MHz, DMSO-d6): δ 12.84 (1H, s H-5), 10.94 (1H, S H-4'), 8.08-6.98 (6H, m H-6, 8, 2', 3', 5', 6'), 6.23 (1H, S H-3), 3.40 (3H, S H-7).

Elemental analysis: Calculated: C, 67.60; H, 4.25; O, 28.14., Found: C, 67.62; H, 4.28; O, 28.17.

ESIMS: *m/z:* 284.9 (M^+).

2.2.5 7-hydroxy-2-(3, 4–dimethoxy phenyl)-4H-chromen-4-one (IVe)

H^1-NMR (400MHz, DMSO-d_6): δ 9.8 (1H, s H-7), 7.66-7.05 (6H, m H-5, 6, 8, 2', 5', 6'), 6.02 (1H, s H-3), 3.45 (6H, s H-3', 4').

Elemental analysis: Calculated: C, 68.45; H, 4.73; O, 26.82., Found: C, 68.42; H, 4.71; O, 26.85.

ESIMS *m/z:* 299.0 (M^+).

2.2.6 2-(4-chlorophenyl)-5-hydroxy-4H-chromen-4-one (IV p)

H^1-NMR (CDCl3, 400 MHz): δ 12.50 (1H, s H-5), 7.84-7.12 (7H, m H-6, 7, 8, 2', 3', 5', 6'), 6.73 (1H, s H-3).

Elemental analysis: Calculated: C, 66.07; H, 3.33; Cl, 13.00; O, 17.60., Found: C, 66.05; H, 3.32; Cl, 12.97; O, 17.63.

ESIMS *m/z*: 274 (M^+)

Spectral data of compounds IV f, IV g, IV h, IVI, IV j is concordance with the literature [13]; similarly compounds IV k, iv n with literature [14] and compounds IV l, IV m, IV o, IV q, IV r with literature [15].

3. CYTOTOXICITY ANALYSIS FOR COMPOUNDS IV a-j

Cellular viability was determined in the presence of test compounds by MTT- micro cultured tetrazolium assay. The cells seeded to flat bottom 96 (10000 cells/100 ul) well plates & cultured in the medium containing 10% serum and allowed to attach and recover for 24 hours in a humid chamber containing 5% CO_2.

MTT (3-(4, 5-dimethylthiazol-2yl)-2,5diphenyl tetrazolium bromide; sigma Catalog noM2128) was dissolved in PBS at 5mg/ml and filtered to sterilize and remove a small amount of insoluble residue present in MTT.

Different concentrations of compounds were added to the cells. After 48 hours, stock MTT solution (10 ul) was added to the culture plate. Cells were again kept in CO_2 incubator for 2 hours. After incubation 100 ul of DMSO was added and mixed.

The absorbance was read at 540 nm by ELISA reader. The results were represented as percentage of cytotoxicity/viability. All the experiments were carried out in triplicates. From the percentage of cytotoxicity the IC50 value calculated.

4. RESULTS AND DISCUSSION

4.1 Chemistry

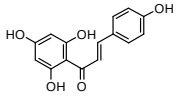
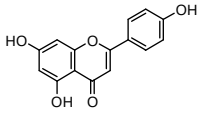
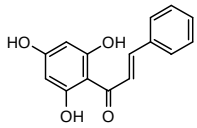
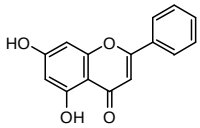
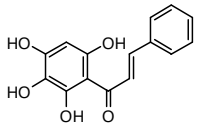
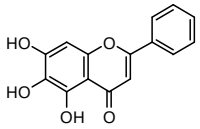
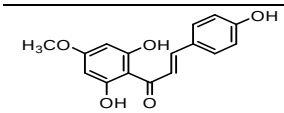
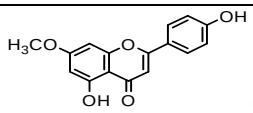
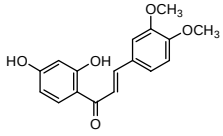
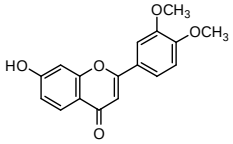
Synthesis of chalcones and flavones using the 2, 4, 6-trimethyl pyridine and potassium iodate for catalytic source under autoclave conditions has been reported. Excellent yields were obtained with in less time. 2, 4, 6-trimethyl pyridine is less toxic than unsubstituted pyridine. The selected catalytic system for the 2nd step is more effective and more eco-friendly. Autoclave conditions are more effective to complete the reaction with in short time with 80-85% of yield.

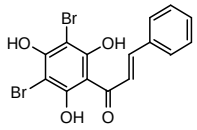
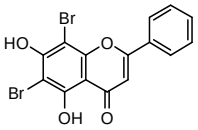
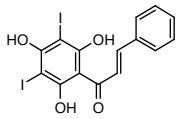
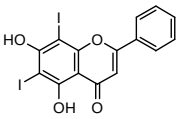
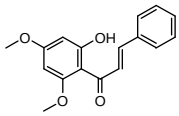
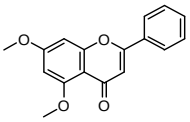
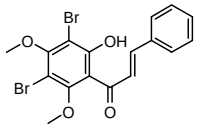
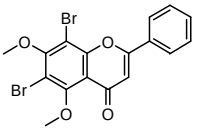
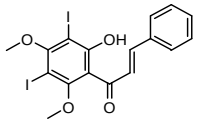
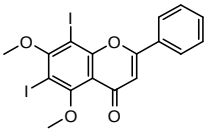
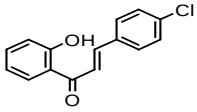
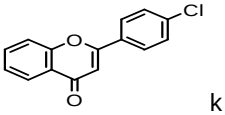
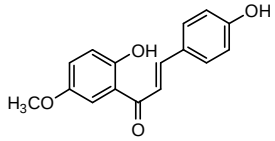
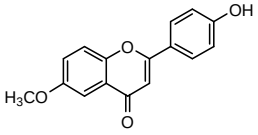
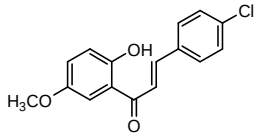
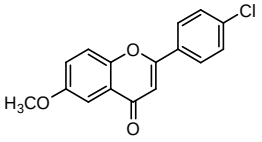
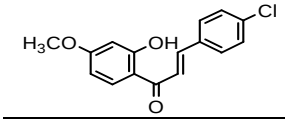
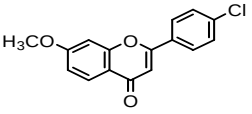
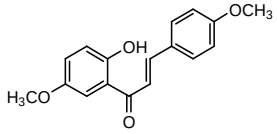
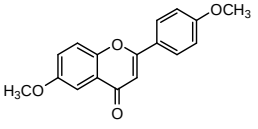
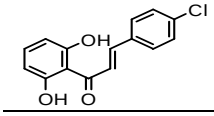
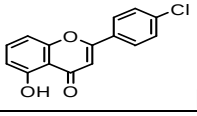
From 1H-NMR spectra of Flavones; ^{1}H due to 3H of pyrone ring is identified by the singlet at 6.16-6.85. The aromatic multiplet appeared at 7.1-7.9. Hydroxy and methoxy protons were identified at around 12.67, 3.40 respectively. Furthermore confirmations were done by peaks in mass spectrums.

4.2 Anti-cancer Activity

Flavone derivatives being considered as efficient anticancer agents, some of flavones were synthesized. Cytotoxic screening was conducted on selective compounds (IVa-j) against A549, human tumour cell lines. The response of tested compounds is good to poor. From this study it reveals that the flavones that contain methoxy groups (compounds d, e and h) show more activity. –OH groups significantly reduce the activity of (compounds a, b, and c). On introduction of –I groups to the flavone nucleus, drastic decrement has been observed (compounds f, g, i and j). The results were tabulated below.

Table 1. Physical data of synthesized compounds

Chalcone (III a-r)	m/z	Flavone (IV a-r)	m.p. ^{0}C	Yield %
	271.27	 a	314-316	82
	255.33	 b	283-285	80
	271.32	 c	284-287	82
	281.21	 d	275-278	81
	299.82	 e	116-118	72

Chalcone (III a-r)	m/z	Flavone (IV a-r)	m.p. ^{0}C	Yield %
	412.62	 f	290-292	84
	507.84	 g	282-285	78
	487.92	 h	132-134	82
	440.16	 i	295-298	80
	535.73	 j	297	75
	260.15	 k	184-186	78
	270.36	 l	110-114	83
	288.85	 m	133-135	80
	289.15	 n	171-174	76
	284.43	 o	152-154	84
	274.19	 p	190-192	73

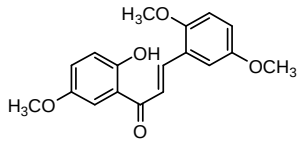
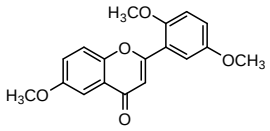
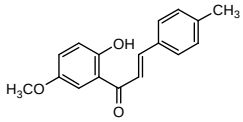
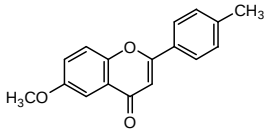
Chalcone (III a-r)	m/z	Flavone (IV a-r)	m.p. ^{0}C	Yield %
	314.38	 q	108-111	78
	268.3	 r	143-146	76

Table 2. Cytotoxic activity

Compound	IC_{50} (µg/ml)
IV a	32.89
IV b	46.82
IV c	46.89
IV d	33.56
IV e	26.35
IV f	53.48
IV g	49.92
IV h	44.32
IV i	55.20
IV j	44.38

5. CONCLUSION

In conclusion, less toxic, more efficient, more yielding catalysts we used under autoclave conditions. Within less time periods only we completed reactions under eco-friendly conditions; thus this method is superior. On screening of cytotoxic activity- it has been concluded that methoxy groups possess more activity, whereas electronegative groups reduce the activity of flavones.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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