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SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL AND CYTOTOXIC EVALUATION OF SOME NOVEL CHALCONES OF 2-FLUOROACETPHENONE

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Abstract:

Chalcones have been considered molecules of great interest considering the multiple methods of synthesis and several starting materials from which they can be synthesized yielding many different molecules. Chalcones are also known to exhibit a wide variety of pharmacological activities and hence are the molecules of great research value in medicinal chemistry. Chalcones also are intermediates for many heterocyclic and other medicinal compounds. In the present research, an attempt was made to synthesize some newer chalcones and evaluate them for their biological activities viz., antibacterial, antifungal activities and cytotoxicity. All the molecules synthesized were characterized and analysed using FTIR and 1H NMR Spectroscopic techniques.

Key words: Chalcones, Antibacterial activity, Antifungal Activity, Cytotoxicity

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INTRODUCTION:

Chalcones, a group of compounds with two aromatic rings connected by a keto-vinyl chain, constitute an important class of naturally occurring flavonoids exhibiting a wide spectrum of biological activities[1]. The presence of a reactive α, β-unsaturated keto functional group is partly responsible for their activity. Chalcones have also been reported to be antiinflammatory, analgesic and antipyretic, bactericidal, antifungal and insecticidal, antimutagenic, chemo preventive activity cardiovascular disease, anticancer activity, cytotoxic activity, antiproliferative activity, antimalarial activity, antiviral activity and anti-HIV activity[2,3,4]. Chalcones are also useful intermediates for the synthesis of several chemical and pharmacological classes of therapeutic agents having heterocyclic structures in them[5,6]. In addition, a number of chalcones with novel substituents were earlier isolated from a number of *Tephrosia* speicies in many studies which were endowed with significant biological activities[7,8]. Based on these observations, it was considered worthwhile to synthesize some new substituted chalcones by Claisen-Schmidt condensation reaction in the present study[9].

MATERIALS AND METHODS:

All the chemicals used in the synthesis were obtained from standard commercial sources. 2-fluoroacetophenone was purchased from Aldrich Chemical Co. (Melwaukee, Wisconsin, USA)

The organic solvents such as methanol, acetone, chloroform and ethyl acetate were of spectral grade used. Anhydrous methanol was obtained by fractional distillation and storing over type 4A molecular sieves. The acetone present in methanol removed by using the following procedure. A mixture of 500 mL of methanol, 25 mL of furfural and 60 mL of 10% sodium hydroxide solution was refluxed for 12 h, then the mixture was distilled and the first few millilitres of the distillate was rejected as it contains trace amount of formaldehyde. Ethanol obtained by distillation of commercial ethyl alcohol was refluxed over ignited calcium oxide for 6 h, distilled at atmospheric pressure, and then used. Some of the solvents were purchased from the local manufacturers and S.D Fine Chem. Ltd, Mumbai, India.

TLC using silica gel-G (Merck grade) as the adsorbent monitored reactions and the solvent systems indicated at appropriate places. Silica gel (100-200 mesh, Merck grade) used for column chromatography. The column subjected to gradient elution using n-hexane, mixtures of hexane and ethyl acetate (5%, 10%, 15%, 25%, 50% and 75% hexane in ethyl acetate), ethyl acetate and mixtures of ethyl acetate and methanol (1%, 2%, 5% and 10% ethyl acetate in methanol). Fractions each of 100 mL collected. The separations of the compounds were checked on TLC under UV lamp and by spraying the plates with 10% sulphuric acid.

All the melting points were determined in open capillaries, using Boitus melting point apparatus, expressed in ^{o}C and are uncorrected.

The ^{1}H NMR spectra of the compounds were recorded either on Bruker AMX 400 MHz or Avance 300 MHz NMR spectrophotometer using TMS as an internal standard and the values are expressed in δ ppm.

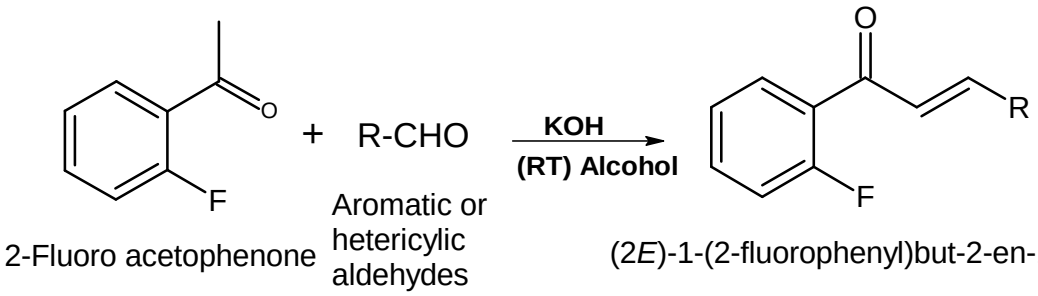
The ^{13}C NMR spectra of the compounds recorded on Bruker AMX 400 MHz NMR spectrophotometer using TMS as an internal standard and the values expressed in δ ppm.

The mass spectra of the compounds were recorded either on Agilent 6100 QQQ ESI mass spectrophotometer method.

Elemental analyses carried out with a Carlo Erba 1108 elemental analyzer. The results of elemental analyses (C, H, N) were within ± 0.4% of the calculated values.

Scheme for synthesis of Chalcones

A mixture of 2-fluoroacetophenone (0.001 mole) and the appropriate aryl aldehyde (0.001 mole) was stirred in ethanol (7.5 mL) and to it aqueous solution of KOH (50%, 7.5 mL) was added. The mixture was kept for 24 h and it was acidified with 1:1 mixture of hydrochloric acid and water, then it was filtered under vacuum and the product was washed with water. The obtained solid purified by column chromatography and recrystallized from a mixture of ethyl acetate and hexane (1:1).



ANTIBACTERIAL ACTIVITY:

The antibacterial activity was tested by determining the minimum inhibitory concentration (MIC) for each compound using serial tube dilution technique[10].

The following organisms were used.

Test organisms:

Gram-positive bacteria:

Staphylococcus aureus (NCIM-2079)

Bacillus subtilis (NCIM-2063)

Gram-negative bacteria:

Escherichia coli (NCIM-2068)

Proteus vulgaris (NCIM-2027)

The antibacterial activity of the chalcones (**C_1** to **C_{15}**) was assessed by determining the MIC, which is defined as the lowest concentration of the compound that completely inhibited the growth of each strain after overnight incubation. MIC values can be determined by a number of standard test procedures. The most commonly employed methods are the tube dilution and agar dilution methods.

ANTIFUNGAL ACTIVITY

The antifungal activity was tested by the same procedure as described in the antibacterial activity, except using Potato-Dextrose-Agar medium[11].

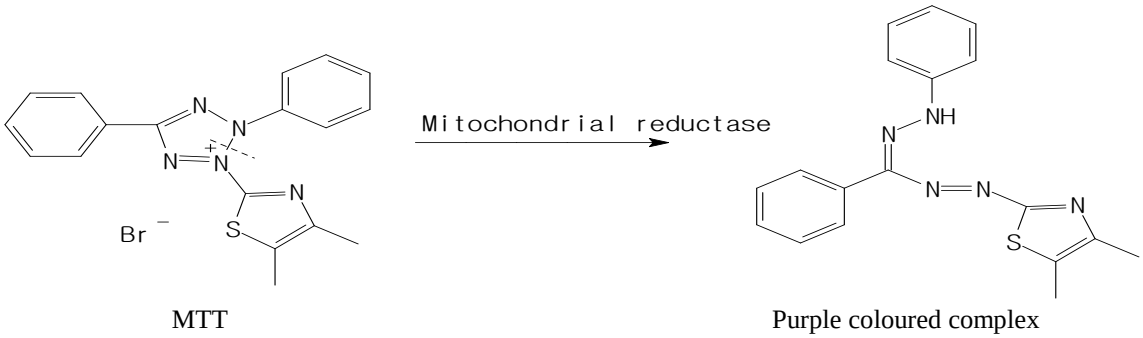
The following organisms were used.

- *Aspergillus* niger (ATCC-6275)
- *Candida tropicalis* (ATCC-1369)

The antifungal activity was tested by the same procedure as described in the antibacterial activity, except using Potato-Dextrose-Agar medium.

CYTOTOXICITY STUDIES:

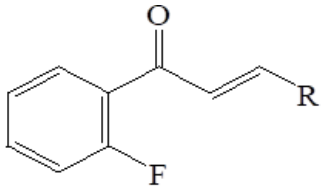
The *in vitro* cytotoxicity of the test compounds (**C_1 to C_{15}**) was evaluated by the MTT assay. This is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passess into the mitrochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilized with DMSO and the released, solubilized formazan reagent is measured spectrophotometrically at 570 nm[12,13]. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells. When the amount of dark purple formazan produced by the cells is treated with a agent compared with the amount of formazan produced by untreated control cells, the effectiveness of the agent in causing death of cells can be deduced through the production of a dose-response curve[14,15].

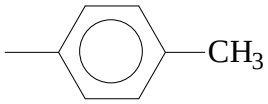
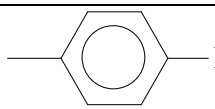
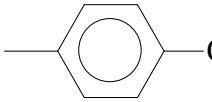
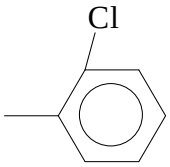
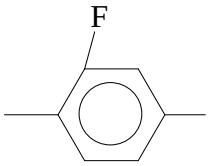
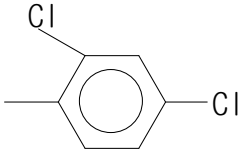
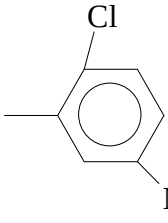
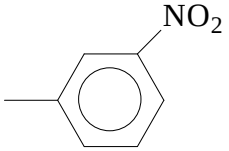
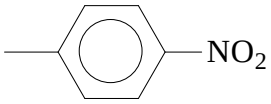


MTT Purple coloured complex

Reduction of MTT

Table 1: Physical characterization data of chalcones (C_1 to C_{15})



Compound	R	Molecular Formula	Relative Molecular Mass (RMM)	Melting Point (ºC)	Yield %
C_1		$C_{16}H_{12}FO$	239	134-137	88
C_2		$C_{15}H_9F_2O$	243	87-90	87
C_3		$C_{15}H_9ClFO$	259	121-124	88
C_4		$C_{15}H_9ClFO$	259	130-133	79
C_5		$C_{15}H_8F_3O$	261	110-113	75
C_6		$C_{15}H_8C_{l2}FO$	292	93-96	92
C_7		$C_{15}H_8ClFNO_3$	304	131-134	82
C_8		$C_{15}H_9FNO_3$	270	114-117	85
C_9		$C_{15}H_9FNO_3$	270	122-125	84

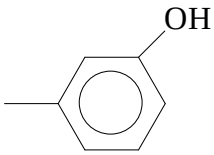
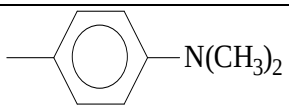
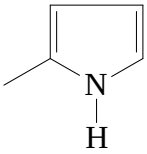
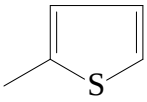
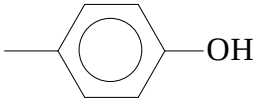
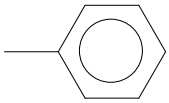
C_{10}		$C_{15}H_{10}FO_2$	241	132-135	93
C_{11}		$C_{17}H_{15}FNO$	268	152-155	86
C_{12}		$C_{13}H_9FNO$	214	101-104	69
C_{13}		$C_{13}H_8FOS$	231	106-109	79
C_{14}		$C_{15}H_{10}FO_2$	241	91-94	86
C_{15}		$C_{15}H_{10}FO$	225	66-69	84

Table 2: IR (KBr disc) spectral data of Chalcones (C_1 to C_{15})

Compound	Position of absoption band (cm^{-1})
C_1	1655 (C=O), 1602 (C=C of Ar), 1505(CH=CH), 925 (C-F)
C_2	1664 (C=O), 1580 (C=C of Ar), 1524 (CH=CH), 928 (C-F)
C_3	1653 (C=O), 1585 (C=C of Ar), 1505 (CH=CH), 835 (C-Cl), 923 (C --F)
C_4	1652 (C=O), 1583 (C=C of Ar), 1502 (CH=CH), 833 (C-Cl), 923 (C-F)
C_5	1655 (C=O), 1581 (C=C of Ar), 1510 (CH=CH), 925 (C-F), 926 (C-F)
C_6	1663 (C=O), 1578 (C=C of Ar), 1506 (CH=CH), 833 (C-Cl), 921 (C-F)
C_7	1658 (C=O), 1603 (C=C of Ar), 1515 (CH=CH), 824 (C-Cl), 1525 (N=O, asymmetric), 1348 (N=O, symmetric), 929 (C-F)
C_8	1655 (C=O), 1605 (C=C of Ar), 1508 (CH=CH), 1533 (N=O, asymmetric), 1345 (N=O, symmetric), 925 (C-F)
C_9	1652 (C=O), 1610 (C=C of Ar), 1502 (CH=CH), 1541 (N=O, asymmetric), 1346 (N=O, symmetric), 923 (C-F)
C_{10}	3520 (O-H), 1648 (C=O), 1612 (C=C of Ar), 1505 (CH=CH), 923 (C-F)
C_{11}	1650 (C=O), 1586 (C=C of Ar),1505 (CH=CH), 1178 (-$N(CH_3)_2$), 921 (C-F)
C_{12}	1652 (C=O), 1605 (C=C of Ar), 1588 (C=N), 1506 (CH=CH), 1375 (C-N), 921 (C-F)
C_{13}	1655 (C=O), 1610 (C=C of Ar), 1505 (CH=CH), 624 (C-S), 923 (C-F)
C_{14}	3460 (O-H), 1648 (C=O), 1606 (C=C of Ar), 1505 (CH=CH), 924 (C-F)
C_{15}	1650 (C=O), 1605 (C=C of Ar), 1502 (CH=CH), 929 (C-F)

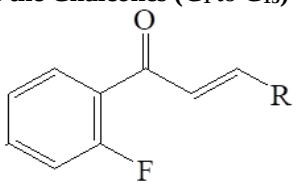
Table 3: ^{1}H NMR spectral data of Chalcones (C_1 to C_{15})

Compound	Chemical shift (δ) in ppm
C_1	2.40 (3H, s, Ar-CH_3), 7.23 (1H, d, *J* = 8.7 Hz, -CO-CH=), 7.73 (1H, d, *J* =8.2 Hz, =CH-Ar), 7.20-7.78 (7H, Ar-H)
C_2	7.15 (1H, d, *J* = 8.5 Hz, -CO-CH=), 7.62 (1H, d, *J* =8.8 Hz, =CH-Ar), 7.05-7.71 (7H, Ar-H)
C_3	7.45 (1H, d, *J* = 8.4 Hz, -CO-CH=), 7.82 (1H, d, *J* =8.6 Hz, =CH-Ar), 7.38-8.20 (7H, Ar-H)
C_4	7.43 (1H, d, *J* = 8.6 Hz, -CO-CH=), 7.80 (1H, d, *J* =8.4 Hz, =CH-Ar), 7.36-8.21 (7H, Ar-H)
C_5	7.40 (1H, d, *J* = 8.3 Hz, -CO-CH=), 7.73 (1H, d, *J* =9.1 Hz, =CH-Ar), 7.15-8.10 (6H, Ar-H)
C_6	7.68 (1H, d, *J* = 8.9 Hz, -CO-CH=), 7.85 (1H, d, *J* =8.2 Hz, =CH-Ar), 7.42-8.20 (6H, Ar-H)
C_7	7.49 (1H, d, *J* = 9.1 Hz, -CO-CH=), 7.65 (1H, d, *J* =8.8 Hz, =CH-Ar), 7.12-8.60 (6H, Ar-H)
C_8	7.40 (1H, d, *J* = 8.4 Hz, -CO-CH=), 7.62 (1H, d, *J* =8.5 Hz, =CH-Ar), 7.20-8.55 (7H, Ar-H)
C_9	7.43 (1H, d, *J* = 8.6 Hz, -CO-CH=), 7.68 (1H, d, *J* =8.8 Hz, =CH-Ar), 7.21-8.59 (7H, Ar-H)
C_{10}	7.38 (1H, d, *J* = 17 Hz, -CO-CH=), 7.52 (1H, d, *J* =8.5Hz, =CH-Ar), 6.89 (1H, s, Ar-OH), 7.18-7.79 (7H, Ar-H)
C_{11}	3.10 (6H,s,-$N(CH_3)_2$, 6.88 (1H, d, *J* = 8.5 Hz, -CO-CH=), 7.75 (1H, d, *J* =8.4 Hz, =CH-Ar), 6.65-7.90 (7H, Ar-H)
C_{12}	7.10 (1H, d, *J* = 9.4 Hz, -CO-CH=), 7.70 (1H, d, *J* =9.4 Hz, =CH-Ar), 6.35-7.90 (7H, Ar-H)
C_{13}	7.12 (1H, d, *J* = 9.6 Hz, -CO-CH=), 7.70 (1H, d, *J* =9.4 Hz, =CH-Ar), 6.62-8.10 (6H, Ar-H)
C_{14}	7.28 (1H, d, *J* = 8.5 Hz, -CO-CH=), 7.59 (1H, d, *J* =9.2 Hz, =CH-Ar), 6.85 (1H,s,Ar-OH),7.21-7.89 (7H, Ar-H)
C_{15}	7.21 (1H, d, *J* = 8.8 Hz, -CO-CH=), 7.62 (1H, d, *J* =8.6 Hz, =CH-Ar), 7.11-7.90 (8H, Ar-H)

Table 4: Antimicrobial activity of chalcones (C_1 to C_{15}) (Expressed as MIC in µg/mL)

Compound	R	*B.subtilis*	*S.aureus*	*E.coli*	*P.vulgaris*	*A.* niger	*C. tropicalis*
C_1	4"-methyl phenyl	128	128	128	128	64	64
C_2	4"-fluoro phenyl	256	256	256	256	256	256
C_3	4"-chloro phenyl	128	256	128	256	64	128
C_4	2"-chloro phenyl	128	128	128	128	64	64
C_5	2",4"-difluoro phenyl	256	256	256	256	128	128
C_6	2",4-dichloro phenyl	64	128	64	64	32	32
C_7	2"-chloro-5"-nitro phenyl	32	64	32	32	16	16
C_8	3"-nitro phenyl	64	64	64	64	32	32
C_9	4"-nitro phenyl	32	64	32	32	32	16
C_{10}	3"-hydroxy phenyl	64	64	64	64	32	32
C_{11}	4"-dimethyl aminophenyl	128	128	128	128	64	64
C_{12}	2"-pyrrolyl	64	64	32	64	32	32
C_{13}	2"-thienyl	64	64	64	64	32	32
C_{14}	4"-hydroxy phenyl	128	128	128	128	64	64
C_{15}	Phenyl	128	128	128	128	64	64
Standard (Ampicillin)		< 1	< 1	< 1	< 1	-	-
Standard Fluconazole		-	-	-	-	< 2	< 2

Table 5: Cytotoxicity of the Chalcones (C_1 to C_{15}) (IC_{50} values in µg/mL)



Compound	R	Cell line		
		HT-29	MCF-7	DU-145
C_1	4"-methylphenyl	76 ± 2	83 ± 1	70 ± 2
C_2	4"-fluorophenyl	90 ± 2	NA	72 ± 1
C_3	4"-chlorophenyl	120 ± 1	172 ± 2	105 ± 2
C_4	2"-chlorophenyl	137 ± 2	183 ± 2	144 ± 1
C_5	2",4"-difluorophenyl	68 ± 2	89 ± 1	43 ± 2
C_6	2",4"-dichlorophenyl	80 ± 2	95 ± 2	61 ± 1
C_7	2"-chloro-5"-nitrophenyl	110 ± 2	NA	101 ± 2
C_8	3"-nitrophenyl	NA	NA	155 ± 2
C_9	4"-nitrophenyl	146 ± 2	167 ± 2	120 ± 1
C_{10}	3"-hydroxyphenyl	164 ± 2	182 ± 2	176 ± 2
C_{11}	4"-dimethylaminophenyl	132 ± 1	117 ± 2	105 ± 2
C_{12}	2"-pyrrolyl	73 ± 2	104 ± 2	87 ± 1
C_{13}	2"-thienyl	42 ± 2	38 ± 1	18 ± 1
C_{14}	4"-hydroxyphenyl	93 ± 2	109 ± 2	76 ± 2
C_{15}	Phenyl	NA	NA	185 ± 2

RESULTS AND DISCUSSION:

A set of 15 chalcones (C_1 to C_{15}) were synthesized and characterized the by using spectral analysis (IR, NMR, Mass), the biological evaluation like anti microbial activity by serial dilution and cytotoxicity by MTT assay method were tested for above compounds.

For anti-microbial activity among the compounds tested, C_7, C_9 with chloronitophenyl moiety, nitrophenyl moiety were found to be the most potent against *B.subtilis*, *E.coli* and *P.vulgaris* having a MIC value of 32 µg/mL in each case. For *S.aureus* the compounds B_7, B_9 with chloronitophenyl moiety, nitrophenyl moiety were found to be the most potent having a MIC value of 64 µg/mL in each case. For anti fungal evaluation among tested compounds, compounds C_7 was found to be the most potent with a MIC value of 16 µg/mL, the componds C_9,C_{12},C_{13},C_{10},C_8 shows potency with a MIC value 32 µg/mL against *A.niger* . In the case of *C.tropicalis* C_7, C_9 showing potency with a MIC value of 16 µg/mL. It is clearly evident that the compound C_7 is more potent than other chalcones derivatives due to presence of more electronegative atoms. The above said results correlated with computational results.

For cytotoxic activity among tested compounds on different cell lines shows promising results. Based on the results concluding that compounds tested against HT-29 cell lines, the compound C_{12} having a pyrrolylphenyl moiety showed maximum activity with a IC_{50} value of 42 µg/mL, against MCF-7 cell lines again the compound B_{21} having the thienyl moiety showed maximum activity (IC_{50} 38 µg/mL), against DU-145 cell lines. It is interesting to note that the compound C_{12} with pyrroylphenyl moiety showed maximum activity IC_{50} 16 µg/mL. It is clearly evident that the compound C_{12} is more potent than other chalcones derivatives due to presence of heterocyclic ring.

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