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Synthesis, Characterization And Anti Bacterial And Cytotoxic Studies Of Novel Chalcones From 2,4 Di Fluoro Acetophenone

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ABSTRACT

Chalcones were an important organic compounds for synthesis of different hetero cyclic compounds contains five and six member nucleus. Most of these compounds are highly bioactive and are widely used in pharmaceuticals. They belongs to an important class of flavonoids, Chalcones are synthesized by Claisen–Schmidt condensation reaction , which involves cross aldol condensation of appropriate aldehydes and ketones by base or acid catalysed reactions followed by dehydration. 13 new Chalcones were synthesized. The structures of newly synthesized compounds were confirmed by spectral evidence. Prepared compounds were tested for anti microbial activity by using serial dilution method and cytotoxicity studies by MTT assay method.

Keywords: Chalcones, Antimicrobial Activity, antifungal activity and cytotoxicity.

INTRODUCTION

Chalcones [1] , a group of compounds with two aromatic rings connected by a keto-vinyl chain, exhibiting a wide spectrum of biological activities. The presence of a reactive α, β unsaturated keto functional group is partly responsible for their activity. Chalcones have also been reported to be analgesic and anti-inflammatory [2-3], anti bactericidal [4], antifungal[5], antiviral activity[6], ant malarial activity[7] , anti mycobacterium activity[8], anticancer activity[9-10], anti platelet activity[11], anti oxidant activity [12] , anti ulcer activity[13]., etc Keeping this broad spectrum of biological activities in mind, in the present investigation it has been considered worthwhile to synthesize some new chalcone derivatives. The compounds were characterized by H^1 NMR and IR analysis. The compounds were tested for their antimicrobial activity by standard protocols.

MATERIALS AND METHODS

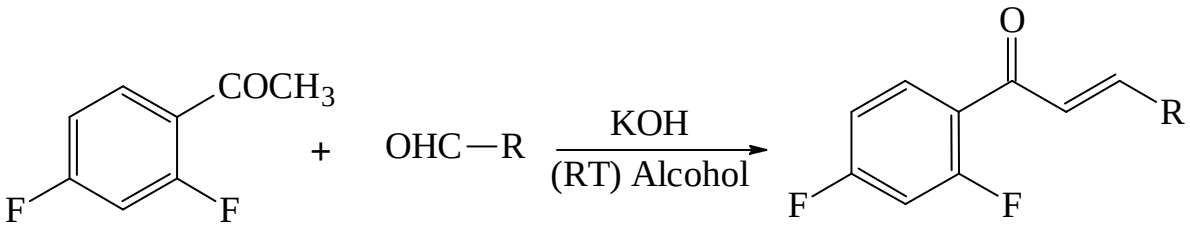
Experimental work: [14-15]

Synthesis of chalcones from 2,4-difluoroacetophenone

General procedure for the synthesis of chalcones (B_1-B_{13})

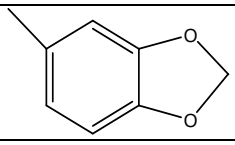
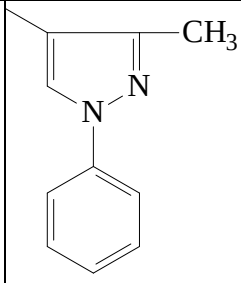
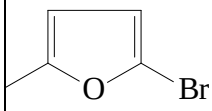
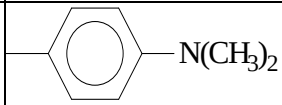
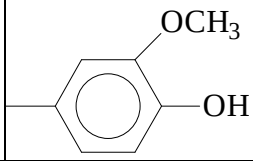
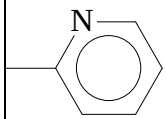
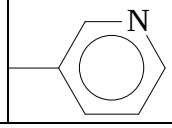
A mixture of 2,4-difluoroacetophenone (0.001 mole) and the appropriate aryl aldehydes (0.001 mole) was stirred in ethanol (7.5 mL) and to it aqueous solution of KOH (50%, 7.5 mL) was added. The mixture was kept for 24 h and it was acidified with 1:1 mixture of hydrochloric acid and water, then it was filtered under vacuum and the product was washed with water.

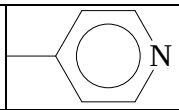
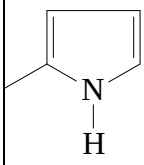
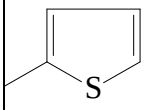
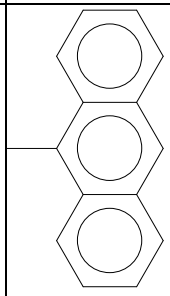
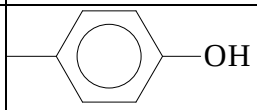
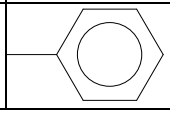
Chemical Reaction:



2,4 Di fluoro Acetophenone Aromatic aldehydes Chalcone derivatives

Table-1 Physical Data For Chalcones From B_1-B_{13}

Compound	R	Molecular Formula	Relative Molecular Mass (RMM)	Melting Point (^oC)	Yield %
B_1		$C_{16}H_{10}F_2O_3$	288	148-151	74
B_2		$C_{19}H_{14}F_2N_2O$	324	112-115	71
B_3		$C_{13}H_7BrF_2O_2$	313	126-129	65
B_4		$C_{17}H_{15}F_2NO$	287	152-155	86
B_5		$C_{16}H_{12}F_2O_3$	290	99-102	89
B_6		$C_{14}H_9F_2NO$	245	91-94	84
B_7		$C_{14}H_9F_2NO$	245	78-81	86

B_8		$C_{14}H_9F_2NO$	245	96-99	89
B_9		$C_{13}H_9F_2NO$	233	101-104	69
B_{10}		$C_{13}H_8F_2OS$	250	106-109	79
B_{11}		$C_{23}H_{14}F_2O$	344	108-111	93
B_{12}		$C_{15}H_{10}F_2O_2$	260	91-94	86
B_{13}		$C_{15}H_{10}F_2O$	244	66-69	84

Spectral data for synthesised chalcones : B_1-B_{13}

1-(2', 4'-difluorophenyl)-3-(3",4"-methylenedioxyphenyl)-2- propen-1-one (B_1)

IR (KBr) (cm^{-1}) : 1643 (C=O), 1574 (C=C of Ar), 1500 (CH=CH), 1240 (O-CH2-O), 929 (C-F), ^{1}H-NMR ($CDCl_3$) ppm : 6.10 (2H,s,-O-CH2O-), 6.88 (1H, d, J = 17 Hz, -CO-CH=), 7.69 (1H, d, J =17 Hz, =CH-Ar), 7.10-7.29 (6H, Ar-H)

1-(2', 4'-difluorophenyl)-3-(1"-phenyl-3"methylpyrazole-4"-yl)- 2-propen-1-one (B_2)

IR (KBr) (cm^{-1}) : 1663 (C=O), 1610 (C=N), 1588 (C=C of Ar), 1510 (CH=CH), 1391 (C-N), 921 (C-F), ^{1}H-NMR ($CDCl_3$) ppm : 2.45 (3H, s, Ar-CH3), 6.85 (1H, d, J = 17 Hz, -CO-CH=), 7.65 (1H, d, J =17 Hz, =CH-Ar), 6.58-7.90 (8H, Ar-H)

1-(2', 4'-difluorophenyl)-3-(5"-bromofuran-2"-yl)-2-propen-1-one (B_3)

IR (KBr) (cm^{-1}) : 1652 (C=O), 1585 (C=C of Ar), 1503 (CH=CH), 929 (C-F), ^{1}H-NMR ($CDCl_3$) ppm : 7.23 (1H, d, J = 17 Hz, -CO-CH=), 7.71 (1H, d, J =17 Hz, =CH-Ar), 7.18-7.95 (5H, Ar-H)

1-(2', 4'-difluorophenyl)-3-(4"-dimethylaminophenyl)-2-propen-1-one (B_4)

IR (KBr) (cm^{-1}) : 1650 (C=O), 1586 (C=C of Ar),1505 (CH=CH), 1178 (-N(CH3)2), 921 (C-F) , ^{1}H-NMR ($CDCl_3$) ppm : 3.10 (6H,s,-N(CH3)2 , 6.88 (1H, d, J = 17 Hz, -CO-CH=), 7.75 (1H, d, J =17 Hz, =CH-Ar), 6.65-7.90 (7H, Ar-H).

1-(2', 4'-difluorophenyl)-3-(3"-methoxy-4"-hydroxyphenyl)-2- propen-1-one (B_5)

IR (KBr) (cm^{-1}) : 3450 (O-H), 1648 (C=O), 1606 (C=C of Ar), 1510 (CH=CH), 1225 (-OCH3), 925 (C-F) , ^{1}H-NMR ($CDCl_3$) ppm : 7.21 (1H, d, J = 17 Hz, -CO-CH=), 7.68 (1H, d, J =17 Hz, =CH-Ar), 7.20-7.93 (6H, Ar-H), 6.75 (1H.s, Ar-OH), 3.82 (3H,s,Ar-OCH3)

1-(2', 4'-difluorophenyl)-3-(2"-pyridinyl)-2-propen-1-one (B_6)

IR (KBr) (cm^{-1}) : 1653 (C=O), 1605 (C=C of Ar), 1595 (C=N), 1508 (CH=CH), 1385 (C-N), 922 (C-F): ^{1}H-NMR ($CDCl_3$) ppm : 7.15 (1H, d, J = 17 Hz, -CO-CH=), 7.65 (1H, d, J =17 Hz, =CH-Ar), 6.30-8.15 (7H, Ar-H)

1-(2', 4'-difluorophenyl)-3-(3"-pyridinyl)-2-propen-1-one (B_7)

IR (KBr) (cm^{-1}) : 1645 (C=O), 1603 (C=C of Ar), 1590 (C=N), 1502 (CH=CH), 1370 (C-N), 923 (C-F); ^{1}H-NMR ($CDCl_3$) ppm : 7.18 (1H, d, J = 17 Hz, -CO-CH=), 7.70 (1H, d, J =17 Hz, =CH-Ar), 7.12-8.20 (7H, Ar-H)

1-(2', 4'-difluorophenyl)-3-(4"-pyridinyl)-2-propen-1-one (B_8)

IR (KBr) (cm^{-1}) : 1650 (C=O), 1605 (C=C of Ar), 1581 (C=N), 1505 (CH=CH), 1373 (C-N), 929 (C-F); ^{1}H-NMR ($CDCl_3$) ppm : 7.15 (1H, d, J = 17 Hz, -CO-CH=), 7.75 (1H, d, J =17 Hz, =CH-Ar), 7.20-8.15 (7H, Ar-H)

1-(2', 4'-difluorophenyl)-3-(2"-pyrrolyl)-2-propen-1-one (B_9)

IR (KBr) (cm^{-1}) : 1652 (C=O), 1605 (C=C of Ar), 1588 (C=N), 1506 (CH=CH), 1375 (C-N), 921 (C-F), ^{1}H-NMR ($CDCl_3$) ppm : 7.10 (1H, d, J = 17 Hz, -CO-CH=), 7.70 (1H, d, J =17 Hz, =CH-Ar), 6.35-7.90 (7H, Ar-H)

1-(2', 4'-difluorophenyl)-3-(2"-thienyl)-2-propen-1-one (B_{10})

IR (KBr) (cm^{-1}) : 1655 (C=O), 1610 (C=C of Ar), 1505 (CH=CH), 624 (C-S), 923 (C-F); ^{1}H-NMR ($CDCl_3$) ppm : 7.12 (1H, d, J = 17 Hz, -CO-CH=), 7.70 (1H, d, J =17 Hz, =CH-Ar), 6.62-8.10 (6H, Ar-H)

1-(2', 4'-difluorophenyl)-3-(9"-anthracenyl)-2-propen-1-one (B_{11})

IR (KBr) (cm^{-1}) : 1658 (C=O), 1605 (C=C of Ar), 1503 (CH=CH), 923 (C-F): ^{1}H-NMR ($CDCl_3$) ppm : 7.35 (1H, d, J = 17 Hz, -CO-CH=), 7.60 (1H, d, J =17 Hz, =CH-Ar), 7.20-8.90 (12H, Ar-H)

1-(2', 4'-difluorophenyl)-3-(4"-hydroxyphenyl)-2-propen-1-one (B_{12})

IR (KBr) (cm^{-1}) : 3460 (O-H), 1648 (C=O), 1606 (C=C of Ar), 1505 (CH=CH), 924 (C-F); ^{1}H-NMR ($CDCl_3$) ppm : 7.28 (1H, d, J = 17 Hz, -CO-CH=), 7.59 (1H, d, J =17 Hz, =CH-Ar), 6.85 (1H,s,Ar-OH),7.21-7.89 (7H, Ar-H).

1-(2', 4'-difluorophenyl)-3-phenyl-2-propen-1-one (B_{13})

IR (KBr) (cm^{-1}) : 1650 (C=O), 1605 (C=C of Ar), 1502 (CH=CH), 929 (C-F); ^{1}H-NMR ($CDCl_3$) ppm : 7.21 (1H, d, J = 17 Hz, -CO-CH=), 7.62 (1H, d, J =17 Hz, =CH-Ar), 7.11-7.90 (8H, Ar-H).

Antimicrobial Activity: Since the chalcones were reported to possess antimicrobial activity, the chalcones prepared during the present work were tested for antibacterial and antifungal activity .

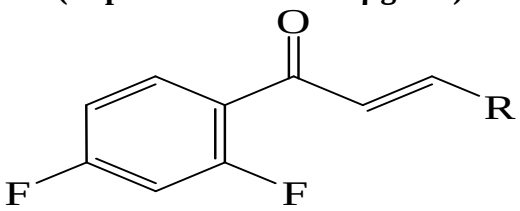
Antibacterial activity: The antibacterial activity was tested by determining the minimum inhibitory concentration (MIC) for each compound using serial tube dilution technique. The following test organisms were used. Gram positive bacteria: *Staphylococcus aureus*, *Bacillussubtilis*,

Gram negative bacteria: *Escherichia coli* ,*Proteus vulgaris*

Antifungal activity The antifungal activity was tested by the same procedure as described in the antibacterial activity, except using Potato-Dextrose-Agar medium. These two organisms were used .*Aspergillus* niger ,*Candida tropicalis*. The results are shown in tables 2 in the case of antibacterial activity and table 3 in the case of antifungal activity.

RESULTS AND DISCUSSION

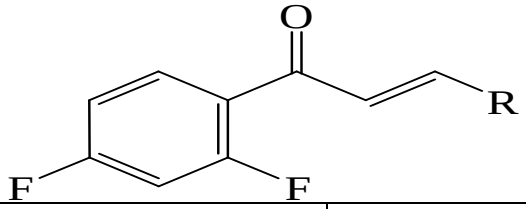
Table 2. Antibacterial activity of synthesised compounds ($B_1$to B_{13}): (Expressed as MIC in µg/mL)



Compound	R	*B.subtilis*	*S.aureus*	*E.coli*	*P.vulgaris*
B_1	3",4"-methylendioxyphenyl	256	128	256	128
B_2	1"-phenyl-3"methylpyrazole-4"-yl	128	128	128	256
B_3	5"-bromofuran-2"-yl	64	64	32	128
B_4	4"-dimethylaminophenyl	64	128	64	64
B_5	3"-methoxy-4"-hydroxyphenyl	128	128	128	128
B_6	2"-pyridinyl	128	256	128	256
B_7	3"-pyridinyl	128	256	256	256
B_8	4"-pyridinyl	128	128	128	128
B_9	2"-pyrrolyl	256	256	64	64
B_{10}	2"-thienyl	128	64	128	128
B_{11}	9"-anthracenyl	256	128	128	256
B_{12}	4"-hydroxyphenyl	256	128	64	64
B_{13}	Phenyl	256	256	256	256

Standard (Ampicillin)		< 1	< 1	< 1	< 1

Table 3. Antifungal activity of synthesised compounds ($B_1$to B_{13}): (Expressed as MIC in µg/mL)



Compound	R	*Aspergillus* niger	*Candida tropicalis*
B_1	3",4"-methylendioxyphenyl	64	128
B_2	1"-phenyl-3"methylpyrazole-4"-yl	64	128
B_3	5"-bromofuran-2"-yl	32	64
B_4	4"-dimethylaminophenyl	64	128
B_5	3"-methoxy-4"-hydroxyphenyl	32	16
B_6	2"-pyridinyl	64	32
B_7	3"-pyridinyl	32	32
B_8	4"-pyridinyl	64	32
B_9	2"-pyrrolyl	64	128
B_{10}	2"-thienyl	64	32
B_{11}	9"-anthracenyl	128	64
B_{12}	4"-hydroxyphenyl	32	32
B_{13}	Phenyl	256	256
Standard Fluconazole		< 2	< 2

Antibacterial activity [14]

From the above results it is evident that all the chalcones synthesized, showed antibacterial activity with different MIC values against the tested organisms, but not comparable with that of the standard. Among the compounds tested, $B_3$and B_4 bromo furany land dimethylaminophenyl moiety was found to be the most potent against *B.subtilis*, *E.coli* and *P.vulgaris* having a MIC value of 64 µg/mL in each case. The chalcones, B_2,B_5-B_8 shows MIC value of 128 µg/mL against *E.coli*, *B.subtilis* and *E.coli* respectively. Some of the chalcones (B_1,B_9, B_{11},B_{12} and B_{13}) showed a MIC of 256 µg/mL against both Gram-positive and Gram-negative bacteria. but most of them showed a MIC value in between 128-256 µg/mL.

Antifungal activity

Among the compounds tested for antifungal activity, compounds B_3,B_5, B_7 to be the most potent with a MIC value of 32 µg/mL against *A.niger* in the case of B_5-B_8,B_{10},B_{12} and against *C.tropicalis* in the case of other two compounds. the compound with electron releasing groups shows moderately the activity.

CYTOTOXICITY STUDIES [14]

The *in vitro* cyto toxicity of the test compounds (**B_1-B_{13})** were performed based on MTT assay method on HT-29 (colon cancer), MCF-7 (breast cancer) and DU-145 (prostate cancer) cell lines. the cell lines were obtained from National Centre for Cell Science (NCCS), Pune, India. Methotrexate was used as reference drug for comparison. Assay was performed in triplicate for three independent determinations. The cytotoxicity was expressed as IC_{50} (µg/mL) which is the

concentration of the compound that inhibited proliferation rate of the tumour cells by 50% as compared to the control untreated cells. IC_{50} values were determined from the plot:% inhibition versus concentration.

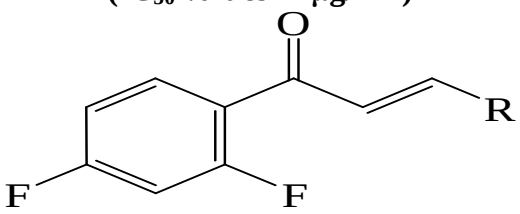
% inhibition at the given concentration = 1- (Absorbance average)

---------- x 100

(Control absorbance average)

IC_{50}=Inv.log(50-c) / m; c and m derived from y=mx+c of plot of % inhibition Vs log C. the results were tabulated .

Table:4 Cytotoxicity of the new chalcones (B_1 to B_{13}).
(IC_{50} values in µg/mL)



Compound	R	Cell line		
		HT-29	MCF-7	DU-145
B_1	3",4"-methelenedioxyphenyl	146 ± 2	153 ± 2	82 ± 2
B_2	1"-phenyl-3"-methylpyrazole-4"-yl	NA	NA	174 ± 2
B_3	5"-bromofuran-2"-yl	67 ± 2	58 ± 2	23 ± 1
B_4	4"-dimethylaminophenyl	132 ± 1	117 ± 2	105 ± 2
B_5	3"-methoxy-4"-hydroxyphenyl	93 ± 2	88 ± 1	74 ± 2
B_6	2"-pyridinyl	NA	148 ± 2	107 ± 2
B_7	3"-pyridinyl	NA	NA	123 ± 2
B_8	4"-pyridinyl	190 ± 2	NA	116 ± 1
B_9	2"-pyrrolyl	73 ± 2	104 ± 2	87 ± 1
B_{10}	2"-thienyl	42 ± 2	38 ± 1	18 ± 1
B_{11}	9"-anthracenyl	NA	NA	186 ± 2
B_{12}	4"-hydroxyphenyl	93 ± 2	109 ± 2	76 ± 2
B_{13}	phenyl	NA	NA	185 ± 2
Methotrexate		12 ± 1	9 ± 1	5 ± 1

Data presented as mean ± SD (n=3). All the compounds and the standard dissolved in DMSO, diluted with culture medium containing 0.1% DMSO. The control cells were treated with culture medium containing 0.1% DMSO. NA- No Activity (i.e IC_{50} > 200 µg/mL)

The prepared chalcones have been evaluated for their cytotoxicity against HT-29 (colon cancer), MCF-7 (breast cancer) and DU-145 (prostate cancer) cell lines. Methotrexate was used as the reference standard. The results clearly revealed that most of the compounds possessed cytotoxic activity as evidenced by the IC 50 values. Of all the compounds tested against HT-29 cell lines, the compounds B_{10} with theinyl (IC_{50} value 42 µg/mL) ,B_3 having bromo furanyl moiety (IC_{50} value 67 µg/mL),B9 having pyrrolyl moiety (IC_{50} value 73 µg/mL) .The other compounds also showed activity but at a higher IC_{50} values. Among the compounds tested for cytotoxicity on MCF-7 cell lines, the compounds B_{10} with theinyl (IC_{50} value 38 µg/mL) ,B_3 having bromo furanyl moiety (IC

$_{50}$ value 58 µg/mL),B_5 having methoxy hydroxyl moiety (IC $_{50}$ value 88 µg/mL) .The other compounds also showed activity but at a higher IC_{50} values. Among the compounds tested for cytotoxicity on DU-145 cell lines, the compounds B_{10} with theinyl (IC $_{50}$ value 18 µg/mL) ,B_3 having bromo furanyl moiety (IC $_{50}$ value 23 µg/mL), B_5 having methoxy hydroxyl moiety (IC $_{50}$ value 74 µg/mL) .The other compounds also showed activity but at a higher IC_{50} values.

CONCLUSION

In all synthesized thirteen Chalcones from the 2,4 di Chloro Acetophenone derivatives B_3,B_4 shows potent anti bacterial activity, B_3,B_5, B $_7$ shows potent anti fungal activity, B_{10},B_3,B_9 shows potent activity against HT-29 (colon cancer), B_{10},B_3,B_5 shows potent activity MCF-7 (breast cancer) and B_{10},B_3,B_5 shows potent activity DU-145 (prostate cancer) cell lines.

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